VOLUME 106 (1) — JANUARY 1997

CUK-H03718-39-P024746

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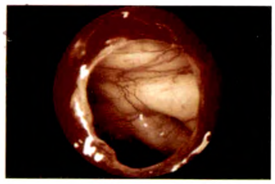

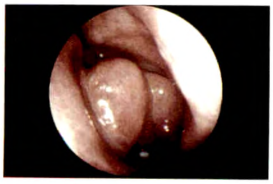

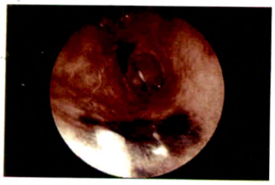

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VOLUME 106 JANUARY 1997 NUMBER 1

# CONTENTS

## *OBITUARY*

**Harold F. Schuknecht** ........ 1

## *ANNALS CLASSIC*

**Reparative Granuloma Following Stapedectomy: A Clinical Entity**
Roger S. Kaufman, MD; Harold F. Schuknecht, MD ........ 5

## *INDEPENDENT CONTRIBUTIONS*

**Treatment of Chronic Postoperative Otorrhea With Cultured Keratinocyte Sheets**
Thomas Somers, MD; Gilbert Verbeken; Stefaan Vanhalle; Bernard Delaey, PhD;
Luc Duinslaeger, MD; Paul Govaerts, MD; Erwin Offeciers, MD, PhD ........ 15

**Sudden Deafness: A Comparison of Anticoagulant Therapy and Carbogen Inhalation Therapy**
Johanna Kallinen, MD; Esa Laurikainen, MD;
Pekka Laippala, PhD; Reidar Grénman, MD ........ 22

**Blood Flow in Common Carotid and Vertebral Arteries in Patients With Sudden Deafness**
Yoshimitsu Ohinata, MD; Kazuo Makimoto, MD; Michiro Kawakami, MD;
Shin-Ichi Haginomori, MD; Michitoshi Araki, MD; Hiroaki Takahashi, MD ........ 27

**Postnatal Change in Angle Between the Tympanic Annulus and Surrounding Structures. Computer-Aided Three-Dimensional Reconstruction Study**
Akihiro Ikui, MD, DMSc; Isamu Sando, MD, DMSc;
Masaharu Sudo, MD; Shuji Fujita, MD ........ 33

**Secretory Otitis Media in Adults: II. The Role of Mastoid Pneumatization as a Prognostic Factor**
Jacob Sadé, MD; Camil Fuchs, PhD ........ 37

**Laryngeal Adjustment in Whispering. Magnetic Resonance Imaging Study**
Koichi Tsunoda, MD; Yasushi Ohta, MD; Yasushi Soda, MD;
Seiji Niimi, MD; Hajime Hirose, MD ........ 41

**Age-Related Changes of Elastic Fibers in the Superficial Layer of the Lamina Propria of Vocal Folds**
Kiminori Sato, MD; Minoru Hirano, MD ........ 44

**Mechanics of Type IV Tympanoplasty: Experimental Findings and Surgical Implications**
Saumil N. Merchant, MD; Michael E. Ravicz, MS; John J. Rosowski, PhD ........ 49

**Three-Dimensional Analysis of Spontaneous Nystagmus in Peripheral Vestibular Lesions**
Andreas Böhmer, MD; Dominik Straumann, MD; Michael Fetter, MD ........ 61

**Immunohistochemical Localization of Manganese Superoxide Dismutase in Rat Vestibular Dark Cell Regions**
Ming-Tang Lai, MD; Takuya Ohmichi, MD, PhD; Satoshi Yorizane, MD;
Kentaro Egusa, MD; Yu Masuda, MD, PhD ........ 69

## *IMAGING CASE STUDY OF THE MONTH*

**Juvenile Cemento-Ossifying Fibroma of the Maxilla**
Erez Bendet, MD; Mati Bakon, MD; Yoav P. Talmi, MD;
Rina Tadmor, MD; Jona Kronenberg, MD ........ 75

## *CLINICOPATHOLOGICAL CONSULTATION*

**Ear Cholesteatoma Versus Cholesterol Granuloma**
Alfio Ferlito, MD; Kenneth O. Devaney, MD; Alessandra Rinaldo, MD;
Christopher M. Milroy, MD; Bruce M. Wenig, MD;
Salvatore Iurato, MD; Brian F. McCabe, MD ........ 79

## *MISCELLANEOUS*

**Book Reviews** ........ 86
**Information for Subscribers** ........ 1a
**Instructions to Authors** ........ 8a
**Index to Advertisers** ........ 12a

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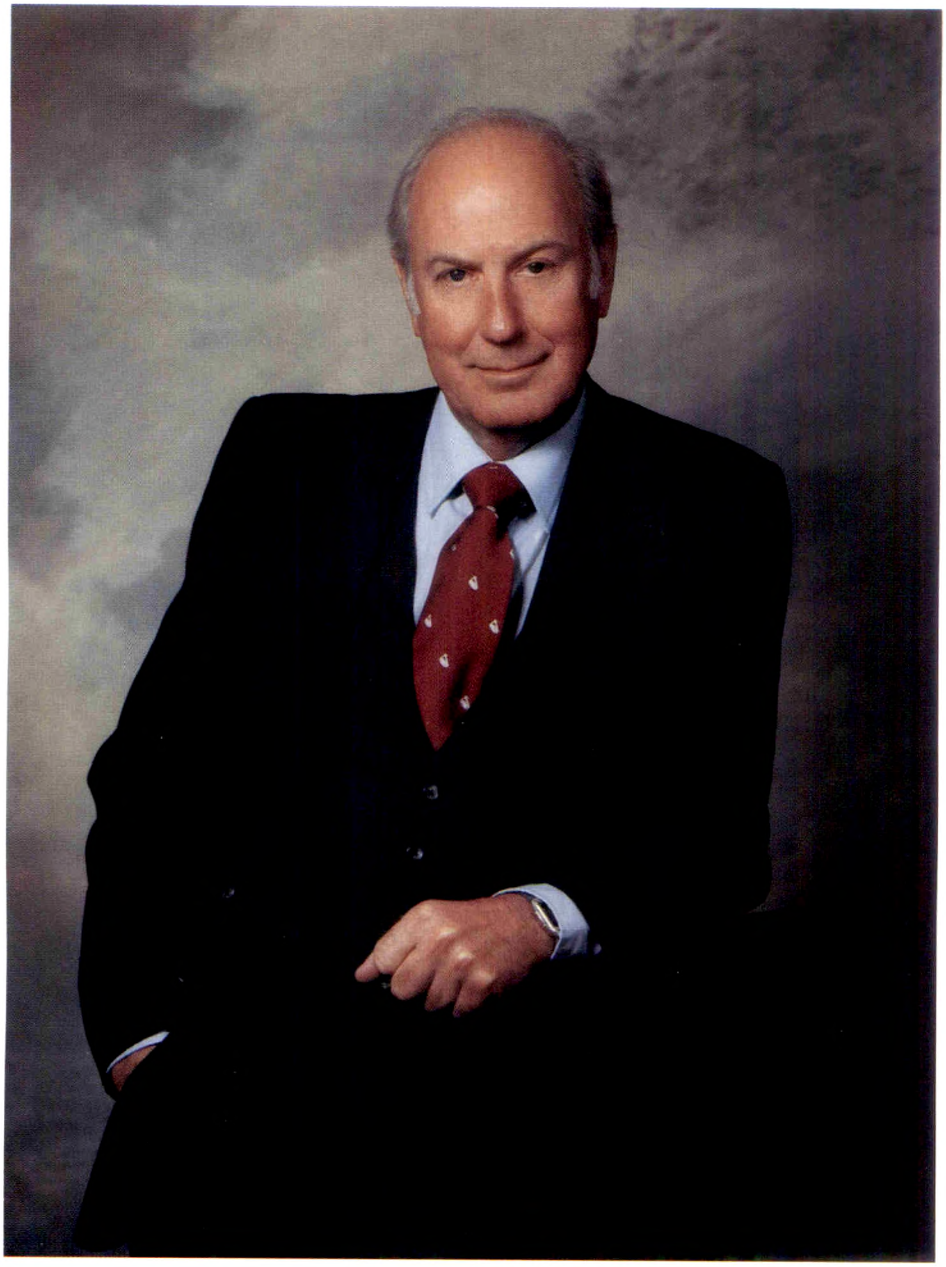

HAROLD F. SCHUKNECHT
1917-1996

*Dr Nadol's thorough summary below documents that Harold Schuknecht can be numbered as one of only a handful of individuals in our specialty whose variety and depth of contributions have had world impact. He possessed one of those rare minds able to cope multidimensionally with analysis and synthesis involving morphology, pathology, physiology, animal experimentation, and physical architectonics (the mental ability to dwell upon complex forms and infer function), thus developing new concepts that he clearly exposited. These attributes he energetically utilized to carry out his roles as author and teacher. In the former he was prolific, and in the latter, selfless. Over a span of four decades he produced a legacy of literature and teaching that will live and stimulate us for many years to come, for, among other reasons, this corpus was carefully honed by self-criticism that few knew of, and was as thoughtful and balanced as his criticism of others.*

*Above all, he was a role model whose magnitude we, his students, may not look upon again. His best memorial, he would agree, would be for us to carry out the work he so carefully delineated to even greater fruition.*

*BRIAN F. MCCABE, MD*

## OBITUARY

# HAROLD F. SCHUKNECHT
## 1917-1996

Harold Frederick Schuknecht, MD, was Professor Emeritus of the Department of Otology and Laryngology at Harvard Medical School and Chief Emeritus of the Department of Otolaryngology at the Massachusetts Eye and Ear Infirmary. Professor Schuknecht was a world-renowned clinical otologist, otopathologist, teacher, and scholar.

He was born on February 10, 1917, in the farming town of Chancellor, South Dakota. He received his undergraduate training at the University of South Dakota and graduated from Rush Medical College at the University of Chicago in 1940. He served a 1-year rotating internship at Mercy Hospital in Des Moines, Iowa, where he met his wife, Anne Bodle. Before residency training, he served 2 years as a general medical officer and 2 years as a flight surgeon with the 15th Air Force in the Mediterranean theater in World War II. He was awarded the Soldier's Medal for his heroic rescue of a pilot who was trapped in a burning B-24. He completed his residency training in otolaryngology at the University of Chicago Clinics in 1949. It was there that he came under the tutelage and influence of Drs John Lindsay, Henry Perlman, Heinz Kobrak, and William Neff, who profoundly influenced his subsequent scientific career.

Dr Schuknecht was an accomplished and innovative otologic surgeon. He started his career as a member of the full-time faculty at the University of Chicago School of Medicine. At that time, his clinical activities were largely confined to head and neck surgery and endoscopy. In 1951, in association with Dr Robert Appleman, he received first prize for an exhibit on the surgical management of carcinoma of the paranasal sinuses at the annual meeting of the American Academy of Ophthalmology and Otolaryngology. His paper on maxillectomy is still a classic. In 1953 he accepted a position as Associate Surgeon at the Henry Ford Hospital in Detroit, Michigan, where he concentrated his clinical work in otologic surgery and pursued basic scientific investigations into the pathophysiology of deafness. He was recruited as the Walter Augustus LeCompte Professor and Chair of the Department of Otology and Laryngology at Harvard Medical School and Chief of Otolaryngology at Massachusetts Eye and Ear Infirmary in 1961, a position he held until retiring from his administrative and clinical activities in 1987. He was among the first surgeons in the United States to perform the modern stapedectomy procedure. He developed and introduced several prostheses for stapes surgery, many of which are still in use worldwide. The innovations and equipment he designed for mastoid tympanoplasty are still in wide clinical use. In 1956 he simplified and perfected transcanal labyrinthectomy for ablating vestibular function in Meniere's disease, and also described the use of intratympanic aminoglycoside therapy for this disorder. He expanded the use of streptomycin by describing its use by the intramuscular route for individuals with bilateral Meniere's disease in 1957 and later in 1970.

In addition to his clinical expertise, Dr Schuknecht was an accomplished and world-recognized investigator in the anatomy, physiology, and pathology of the ear. His early research work included the determination of auditory thresholds in experimental animals and the use of behaviorally conditioned animals in a series of classic experiments, including the study of traumatic hearing loss, the behavioral effects of partial section of the auditory nerve, and apical lesions of the cochlea. While at Henry Ford Hospital, he and his associate Dr John Churchill demonstrated that cholinergic nerve fibers were present in the organ of Corti and that these were probably of efferent origin from the olivocochlear bundle. He also demonstrated a system of channels, the "canaliculi perforantes Schuknechtii," in the osseous spiral lamina, by which perilymph comes in direct continuity with the neural supply of the ear and basal poles of hair cells. Other experiments demonstrated the patency of the cochlear aqueduct sufficient to allow passage of red blood cells, the independent origin of endolymph in the auditory and vestibular systems, and the pathologic effects of fistulae of the cochlear duct. At Massachusetts Eye and Ear Infirmary he significantly expanded his research activities. With the collaboration of his good friend Dr Robert Kimura, the Electron Microscopy Laboratory was developed. In addition, Dr Schuknecht supported the research efforts of the Eaton Peabody Laboratory, which had very recently been established under the direction of Dr Nelson Kiang. Dr Schuknecht's academic accomplishments were described in detail by Beecher and Altschule in their book.[1]

The principal focus of Dr Schuknecht's research work at Harvard, both during his tenure and following professional retirement, was the importance of the underlying anatomy and pathology of the ear to the understanding of disorders of the ear. The clinical problem was underlined by the quote from Joseph Toynbee with which Schuknecht chose to begin the second edition of his text[2]: "If we carefully survey the history of the rise and progress of aural (surgery), as a distinct branch of scientific surgery, one main cause of the disrepute into which it has fallen may be traced to the neglect of the pathology of the organ of hearing."[3] His interest in temporal bone anatomy and pathology began under the tutelage of Dr John Lindsay. The study of otopathology and his active and innovative clinical practice thus became a logical continuity. It can be said that Dr Schuknecht reestablished the histologic and scientific basis for modern medical and surgical otologic intervention, based on his lifelong study and documentation of human temporal bones. His contributions to this area are many, but of particular interest were studies of otosclerosis, Meniere's disease and other vestibular disorders, and presbycusis. Examples of this fruitful marriage between otopathology and clinical practice were many. In 1962 he described his concept of positional vertigo based on sediment of high specific gravity on the cupula of the posterior semicircular canal. In association with his colleague Dr Robert Kimura, he demonstrated that obstruction of the endolymphatic sac in experimental animals produces endolymphatic hydrops similar to that seen in human Meniere's disease. In the following years there were several articles describing the effects of Meniere's disease in the human, including rupture and healing of inner ear membranes and degeneration of the apical spiral ganglion. On the basis of his earlier research experience in Chicago, he was the first to develop an auditory frequency map for the human. This he developed and perfected over the years in conjunction with his logical and convincing categorization of subtypes of presbycusis, namely, sensory and neural degeneration, atrophy of the stria vascularis, and degenerative changes in the supporting structures of the inner ear. As a clinician-scholar, Schuknecht was never content with simply descriptive pathology. Instead, he was always searching for clues to the pathophysiology of disease processes. The second edition of his magnum opus, *Pathology of the Ear*,[2] was completed in 1993 and will remain a classic in otopathology, and at the same time provides fundamental information for every otologic surgeon. His scholarly career includes the publication of over 300 original articles, editorials, and reviews, and seven books devoted to anatomy, pathology, and surgery of the ear.

In addition to his clinical expertise, Schuknecht was a willing and gifted teacher. His temporal bone collection established at the Massachusetts Eye and Ear Infirmary now contains over 1,500 sets of well-documented specimens. This collection and his willing expert mentorship attracted residents and postgraduate students from many nations. Students honored him in 1973 by the establishment of the International Otopathology Society, also known as the Schuknecht Society. Although originally starting with former research fellows, the Society now includes the students of former fellows and others with a serious interest in human otopathology. The Society has more than 120 members from 30 countries, who meet in scientific sessions every 3 years. In his teaching career he quickly distinguished himself as a no-nonsense individual. He required all residents in otolaryngology to attend "Sunday school" for a review of recent otopathologic cases and was among the first chairmen to insist all residents be involved in research projects. Many of his former residents and fellows are now chairmen of departments of otolaryngology worldwide.

During his professional career, Dr Schuknecht received many prestigious awards, including the Award of Merit from the Association for Research in Otolaryngology, the Shambaugh Prize in Otology from the Collegium Otorhinolaryngologicum Amicitiae Sacrum, the Award of Merit from the American Otological Society, Inc, a Presidential Citation from the American Laryngological, Rhinological and Otological Society, Inc, and the Distinguished Award for Contributions in Clinical Otology from the American Academy of Otolaryngology–Head and Neck Surgery. He served as president of the New England Otolaryngological Society, the Eastern Section of the American Laryngological, Rhinological and Otological Society, Inc, the American Otological Society, Inc, and the American Neurotology Society. He was a member of the editorial boards of *Acta Oto-Laryngologica; Annals of Otology, Rhinology & Laryngology; European Archives of Oto-Rhino-Laryngology; American Journal of Otolaryngology; Otolaryngology–Head and Neck Surgery;* and *Laryngoscope*. He was a member of 16 professional societies and an honorary member or fellow of many more, including the Royal Society of Medicine of London, the Royal College of Physicians and Surgeons of Glasgow, and the Royal College of Surgeons of Edinburgh.

Although there seemed to be little leisure time, Hal knew how to have fun: traveling, golfing, fishing, videotaping the Boston Marathon, and finding time for his many friends.

Medicine in general and the specialty of otology in particular has lost a giant, and many, many of us have lost a marvelous colleague and good friend. Through his meticulous writings and scientific collections and the training of hundreds of fellows, residents, and students, his clinical and scientific contributions will continue to influence scientific inquiry and the practice of otology.

JOSEPH B. NADOL, JR, MD
Submitted Oct. 29, 1996

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*This article originally appeared in December 1967, Volume 76, No. 5, pages 1008-1017, and is reproduced here in its entirety.*

# REPARATIVE GRANULOMA FOLLOWING STAPEDECTOMY:

## A CLINICAL ENTITY

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The formation of reparative granuloma as one of the causes for sensorineural hearing loss as a complication of stapedectomy has been reported previously.[6,13,17] The purpose of this paper is to present our experiences with this problem and to show that this condition can be diagnosed and treated with good results in many cases.

The incidence of sensorineural loss from all causes following stapedectomy was reported in several studies[8,9,11] to range from 0.6 per cent to 3 per cent. Harris and Weiss, on the other hand, found six granulomas which were confirmed by surgery, in 119 stapedectomies for a 5 per cent incidence.

We found ten granulomas from a total of 780 recent stapedectomies for an incidence of 1.3 per cent. Three of these granulomas occurred following 170 fat-wire implants and seven occurred after 610 Gelfoam®-wire implants. All of these granulomas were grossly and microscopically confirmed.

The evidence indicates that reparative granuloma is a major cause of sensorineural hearing loss following stapedectomy. During the period of this study there was only one additional case of sensorineural hearing loss in this series of 780 stapedectomies. Tympanotomy in this case disclosed that a perilymph fistula occurred after a Gelfoam-wire implant.

### INITIAL SIGNS AND SYMPTOMS

Of the ten cases of poststapedectomy granuloma, nine developed symptoms within the first six weeks (Table I). One patient experienced the onset of symptoms ten months following surgery.

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From Massachusetts Eye and Ear Infirmary, Boston, and the Department of Otology and Laryngology, Harvard Medical School.

TABLE I

TIME OF ONSET

| *Time (in weeks)* | *Number of cases* |
|---|---|
| One | 2 |
| Two | 3 |
| Three | 2 |
| Four | 1 |
| Six | 1 |
| Forty | 1 |
| TOTAL | 10 |

Four of the ten patients experienced a sudden hearing loss after an initial hearing gain, and three had a gradual hearing loss occurring over a period of two or three days. Three experienced vertigo as the first symptom (Table II). None of these patients complained of pain.

TABLE II

INITIAL SYMPTOM

| *Symptom* | *Number of cases* |
|---|---|
| Sudden hearing loss | 3 |
| Gradual hearing loss | 3 |
| Vertigo | 2 |
| Distortion of sounds | 1 |
| No hearing improvement | 1 |

Audiometric studies revealed a pure tone threshold loss for bone conduction with little or no conductive component (Table III). Seven of the ten cases exhibited a marked decrease in speech discrimination scores.

TABLE III

AUDIOLOGICAL FINDINGS PRIOR TO TYMPANOTOMY

| *Results* | *Number of cases* |
|---|---|
| Threshold elevation for bone conduction | 10 |
| Associated conductive loss | 1 |
| Speech discrimination scores | |
| 60 - 100% | 3 |
| 60% or less | 7 |

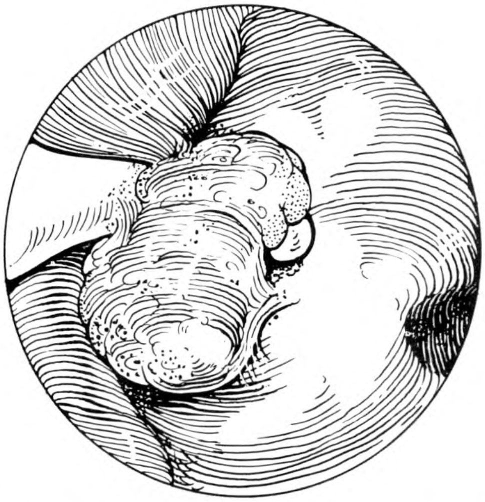


Fig. 1.—Artist's sketch of granuloma in the middle ear.

Examination of the tympanic membranes at the time of onset of symptoms usually revealed a reddish-grey discoloration most obvious in the posterosuperior region. There was no evidence of a fluid line.

## PATHOLOGICAL FINDINGS

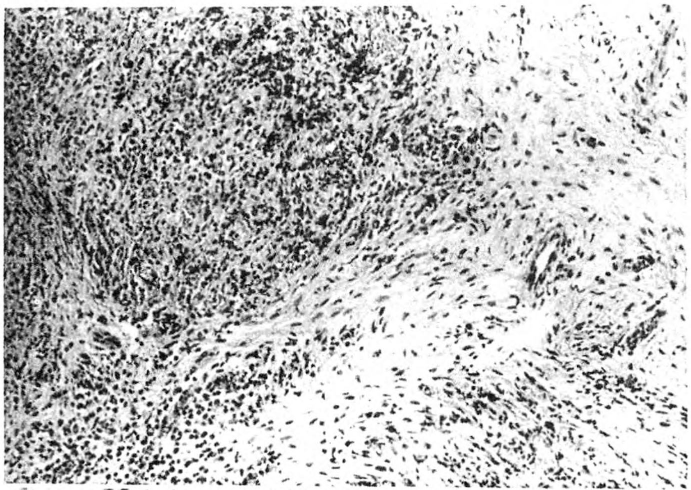
A consistent finding at the time of surgical exploration was a red, soft vascular mass extending from the oval window niche to the tympanic membrane encompassing the prosthesis and long process of the incus (Fig. 1). In two, the granulomatous mass also extended into the vestibule. When a Gelfoam-wire implant had been used, a layer of Gelfoam frequently was found beneath the granuloma, appearing to be partly resorbed, with the remaining portion depressed slightly into the vestibule.

Microscopic examination of the granuloma removed four to six weeks after fat-wire implants (Fig. 2) revealed fibroblastic proliferation and marked invasion with acute and chronic inflammatory cells with adipose tissue still present. Foreign body giant cells also were seen. The fat graft granuloma removed ten months poststapedectomy revealed less inflammation but more fibrous tissue and a surface covering of middle ear mucosa.

The granulomas which followed Gelfoam-wire implants consisted of young granulation tissue characterized by loosely arranged fibroblasts, numerous capillaries, and areas of lymphocytic infiltration. Very few polymorphonuclear leucocytes were present. Occasional foreign body giant cells could be seen.

## ETIOLOGY

The term granuloma implies a tumor-like mass of granulation tissue, i.e., actively growing fibroblasts, fibrous tissue, capillary buds, occasional foreign



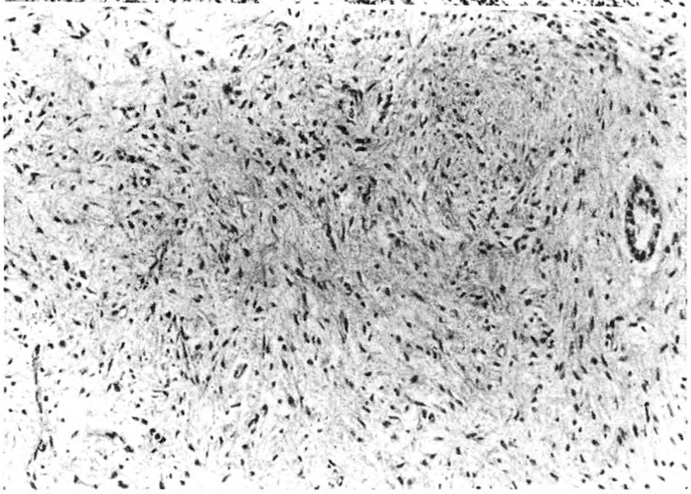


Fig. 2.—Granuloma, removed six weeks poststapedectomy and fat-wire insert.
Fig. 3.—Granuloma removed six weeks poststapedectomy and Gelfoam-wire insert.

body giant cells, clusters of round cells and varying numbers of polymorphonuclear leucocytes. Unfortunately, the precise causative agent in most inflammatory conditions cannot be identified from the morphological picture.[18]

Kylander and Schuknecht[12] studied the sequential morphological changes in the oval window area in the squirrel monkey following stapedectomy and Gelfoam implant. The initial response to the Gelfoam was one of acute inflammation with an abundance of polymorphonuclear leucocytes. After 48 hours, the Gelfoam was beginning to degenerate but no fibroblasts were present. By one week, fibroblasts and fibrous tissue were in abundance; however, polymorphonuclear leucocytes were still present. At the end of two weeks, the microscopic picture most closely resembled that of the granuloma. The tissue consisted of

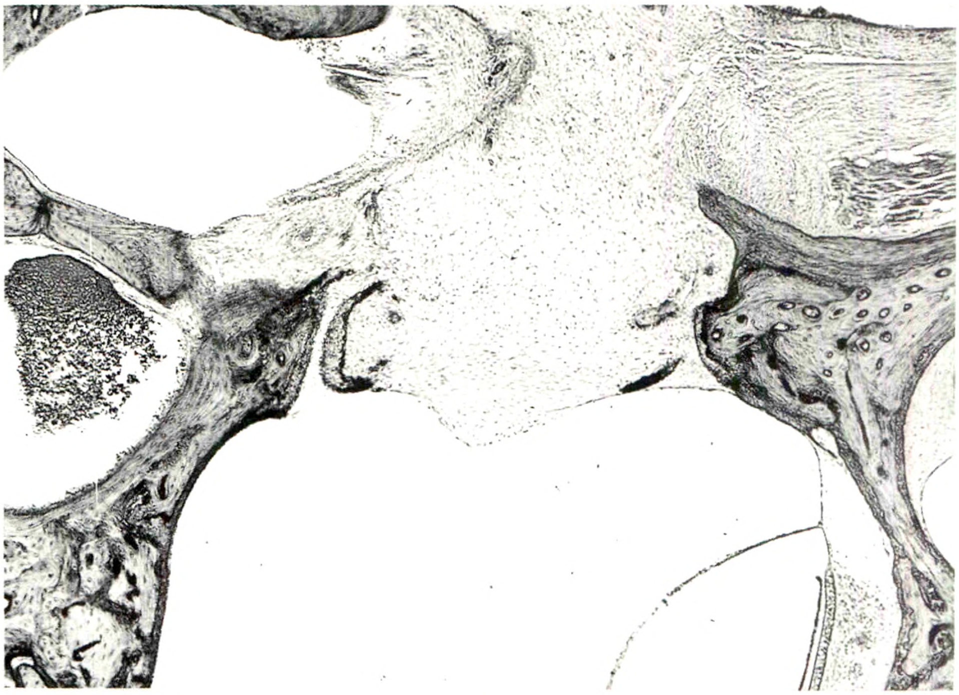


Fig. 4.—Normal reparative process following Gelfoam replacement of footplate in the squirrel monkey. Two weeks postoperative. (From Kylander and Schuknecht)[12]

fibroblasts, some lymphocytes, foreign body giant cells, and increased vascularity (Fig. 4).

As this reparative process resembles the cytological structure of granulation tissue (Fig. 3), one might conclude that the granuloma occurring in these patients is an exaggeration of the normal reparative process. The repair of any injured tissue is governed by the regenerative capacity of the affected cells and the proliferative activity of connective tissue that fills the defect. These findings in animals show that a small granulomatous reaction is to be expected as a normal sequel to the Gelfoam-wire insert; however, this should be differentiated from the more severe overabundant granulomatous formation occurring in the patients with poststapedectomy hearing loss

In a previous report, Schuknecht[21] showed that granulomas may follow stapedectomy and fat-wire insert, and it was hoped that the use of the Gelfoam-wire insert would alleviate this problem; however, it appeared that the reparative granuloma occurred as frequently with Gelfoam-wire as with fat-wire inserts. Thus, we cannot incriminate the specific graft material as the etiologic agent.

There was no evidence that gross infection played any role as a causative agent in the formation of the granuloma in these ten cases. Otitis media was never apparent. All patients received one gram of tetracycline orally per day beginning on the day of surgery and continuing for seven days thereafter.

Rock[19] recently reported the occurrence of two middle ear granulomas related to surgeons' glove powder. We have reviewed the microscopic slides of our granulomas using polarized light and could find only one starch granule present in all the granulomas. We feel that starch was not a causative factor in our cases of middle ear granuloma. Every effort was made to avoid contact between the surgeons' and nurses' gloves and the prosthesis and graft material. All of the Gelfoam-wire inserts used in this series were prefabricated and sterile packaged and handled only with surgical instruments.

The occurrence of the granuloma does not seem to be determined by the surgical technique, since granulomas occurred in some ears with a minimum of surgical trauma. The following case history is illustrative of the occurrence of granuloma following an uncomplicated surgical procedure. Unfortunately, there was some delay in removal and a permanent hearing loss resulted.

Mrs. A.C., a 49 year old female, experienced a progressive bilateral hearing loss of ten years' duration. Audiometry showed bilateral conductive hearing loss and speech discrimination scores of 96 per cent in both ears.* A right stapedectomy procedure was performed and a thin footplate was encountered with an anterior marginal otosclerotic focus. A 1 mm opening was made in the center of the footplate and a prefabricated (McGee) steel piston was inserted. There was slight bleeding into the vestibule. Fatty connective tissue was placed around the shaft of the piston. The postoperative course was uneventful and audiometric tests revealed elimination of the conductive component of the hearing loss.

One year later, a left stapedectomy was performed and a similar thin footplate with anterior marginal otosclerosis was encountered. It was removed completely with no bleeding into the vestibule. A prefabricated Gelfoam-wire prosthesis was inserted without difficulty and the immediate postoperative course was uneventful with a good subjective hearing gain. Twenty days after surgery, on awakening in the morning the patient experienced vertigo and diminished hearing in the left ear. An exploratory tympanotomy performed five days later revealed the oval window to be filled with a soft mass of granulation tissue. This tissue had not invaded the vestibule, which was found to contain clear fluid. The granuloma was removed in its entirety and a fat-wire prosthesis was inserted. Audiometric tests made three weeks following removal of the granuloma disclosed a speech reception level of 38 decibels and the speech discrimination score of 44 per cent in the left ear. Three years later, the speech reception was 56 decibels and the discrimination score was 60 per cent. There was no conductive component to the hearing loss.

Experimental studies in animals[7,16] show that serous labyrinthitis as manifested by mild endolymphatic hydrops and fibrillar precipitate in the perilymph-

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* Pure tone audiometric values are based on the ISO calibration and the speech discrimination represents the PB-Max score on 50 words presented by live monitored voice.

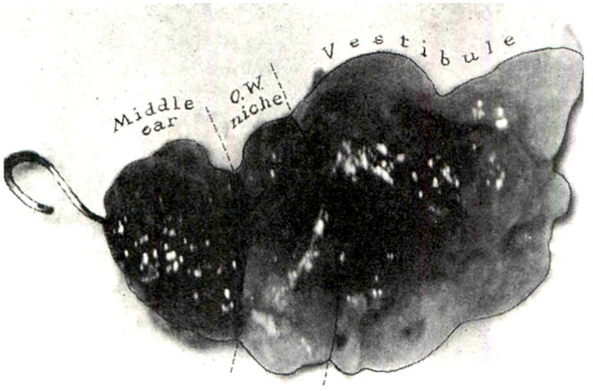

Fig. 5.—Granuloma, removed 13 days poststapedectomy and Gelfoam-wire insert. Note large mass which protruded into the vestibule. (Courtesy of Dr. R. Gacek)

atic fluid can follow stapedectomy. The process is usually reversible; however, it can progress into serofibrinous reaction with degeneration of the organ of Corti. The severity of the serofibrinous labyrinthitis can vary from a mild fibrous tissue invasion of the vestibule to more extensive invasion of the scala vestibuli. Apparently the granuloma formation in human ears following stapedectomy is associated with a serous labyrinthitis in the early stages, at which time it may be reversible by surgical intervention, and by serofibrinous labyrinthitis in the later stages, at which time varying degrees of permanent hearing loss are inevitable.

## TREATMENT AND PROGNOSIS

The evidence suggests that the inner ear reaction occurring with reparative granuloma can be arrested or reversed by timely surgical intervention. The sooner the granuloma is removed, the more likely the possibility of avoiding the irreversible stage of serofibrinous labyrinthitis.

The method of treatment we followed was to remove the granuloma in its entirety including the prosthesis, and to introduce a different type of graft material. For example, if the initial insert consisted of fat-wire, the replacement consisted of Gelfoam-wire and vice versa. In two of the ten cases, the granulomatous tissue bulged into the vestibule. We found, like Pratt and Winchester, that the mass was not adherent to the saccule or utricle and could be removed from the vestibule.

The advisability of removing the granuloma completely was further confirmed in a case encountered recently by one of our associates (Dr. Richard Gacek).* This 50-year-old patient with bilateral otosclerosis underwent an uncomplicated right stapedectomy and a Gelfoam-wire replacement. Thirteen days later, after an initial subjective hearing improvement, the patient experi-

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* This case is not included in our statistics.

TABLE IV

HEARING AFTER TYMPANOTOMY

| *Results* | *Number of cases* |
|---|---|
| Complete recovery | 2 |
| Partial recovery | 3 |
| No recovery | 3 |
| Further regression | 2 |

enced a hearing loss and mild vertigo. Audiometric tests revealed a 75-decibel pure tone threshold loss of the sensorineural type with a discrimination score of 28 per cent. Surgical intervention four hours after the onset of symptoms revealed a large granuloma in the oval window niche enveloping the prosthesis and long process of the incus and invading the vestibule. It was removed in its entirety. Most of the granuloma was removed in one piece; however, several additional small pieces were removed from the vestibule with a small hook. Postoperatively there was a gradual improvement in hearing over a three-week period and audiometric tests taken four weeks later showed return of pure-tone bone conduction thresholds to the prestapedectomy levels and a speech discrimination score of 88 per cent (Fig. 5).

The results of surgical removal of the granulomas are shown in Table IV. Of these ten cases in our series, five had improvement in their hearing; three had no change and two had further loss. The latter two cases retained some useful hearing and normal caloric responses. Probably all patients benefited from the surgery, for five had hearing recovery and the remaining five retained hearing which otherwise may have been lost.

The following history of one of our cases further illustrates the benefit that may be accomplished by prompt surgical removal of the granuloma. R.K., a 37-year-old physician, had experienced a progressive bilateral hearing loss of three years' duration. Audiometry disclosed a pure sensorineural hearing loss on the left and a mixed loss on the right. The speech reception threshold on the right was 26 decibels and the speech discrimination score was 100 per cent. A partial right stapedectomy was performed in which two-thirds of the footplate was removed, leaving a dense anterior otosclerotic focus, and was followed by insertion of a Gelfoam-wire prosthesis. The immediate postoperative course was uneventful. Upon removal of the packing from the external auditory canal on the seventh day, his hearing was subjectively greatly improved. On the ninth postoperative day, he noticed a sudden deterioration of hearing on the right side. Audiometric testing revealed a severe sensorineural loss: the speech reception threshold was 50 decibels and the speech discrimination score was 56 per cent. A tympanotomy performed eight hours after the onset of symptoms revealed a large granulomatous mass filling the oval window niche, enveloping the prosthesis and the long process of the incus. The granuloma did not extend into the vestibule. The entire granuloma and prosthesis were removed and a fat-wire prosthesis was inserted. Two weeks later, pure tone thresholds for air conduction for the speech frequencies averaged 16 decibels, and the speech discrimination score was 100 per cent. He continued subjectively to have excellent hearing in this right ear (six-month follow-up).

## CONCLUSIONS

For several years reparative granuloma has been recognized as a complication of stapedectomy. Our recent experience with this entity indicates that it can be diagnosed early in its development, and that it constitutes a surgical emergency.

Granulomas accounted for a 1.3% incidence of sensorineural hearing loss in the present series of stapedectomies. The usual clinical manifestations are sudden or gradual hearing loss sometimes with vertigo, occurring between one and six weeks after an initial hearing improvement. Examination of the tympanic membrane usually reveals a reddish-grey discoloration most obvious in the posterosuperior region. Audiometric tests show a hearing loss for bone conduction and deterioration in speech discrimination.

Surgical exploration reveals a large granuloma filling the oval window and enveloping the prosthesis and long process of the incus and sometimes invading the vestibule. Microscopically this tissue consists of granulation tissue, i.e., fibrous tissue, fibroblasts, capillary buds, lymphocytes, and foreign body giant cells.

The etiology of the granuloma is unknown; however, it resembles the proliferative reaction occurring in a normal reparative process. It does not appear to be related to the graft material used, the surgical technique, starch glove powder or to infection.

The inner ear changes resulting from the granuloma most probably represent those of a serous labyrinthitis progressing to a serofibrinous labyrinthitis.

Surgical exploration of ten cases of poststapedectomy reparative granuloma with removal of the granuloma and replacement with a different prosthesis and graft material resulted in recovery of auditory function in five cases and preservation of some useful function in the other five. On the basis of previous experience with sudden hearing loss following stapedectomy, we believe most of these ten patients would have experienced severe and permanent hearing losses had surgery not been performed.

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*Ann Otol Rhinol Laryngol* 106:1997

# TREATMENT OF CHRONIC POSTOPERATIVE OTORRHEA WITH CULTURED KERATINOCYTE SHEETS

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Cultured allogeneic ear keratinocyte sheets were used to treat 26 ears presenting with long-standing (average 37 months) chronic otorrhea, resistant to regular treatment, long after surgery for atresia (n = 8), cholesteatoma (n = 10), and chronic otitis media (n = 8). Complete epithelial healing and cessation of otorrhea were obtained in 18 cases (69%), following an average of 2.2 weekly applications. Temporary epithelial healing lasting at least 3 months was observed in 3 patients (12%) subsequently needing repeated applications. Lack of complete epithelialization was documented in 5 cases (19%). In 3 of those 5 cases, a reason could be determined. The authors speculate that the allocultured keratinocytes are able to promote migration and proliferation of resident cells at the wound edges, despite their short survival time, by release of keratinocyte-stimulating factors.

KEY WORDS — cytokine, growth factor, keratinocyte, middle ear surgery, otorrhea, tympanoplasty.

## INTRODUCTION

Chronic otorrhea from operated ears remains a troublesome complication, most often seen after radical mastoid surgery[1] or surgery for atresia,[2] but occasionally also after routine tympanoplasty.[3]

Epithelialization of the tympanic graft and (if opened) of the mastoid cavity is usually observed within the first 2 to 3 months after surgery, antibiotic ear drops being given to prevent secondary infection of the exudate and of the epithelial and mesenchymal outgrowths originating from the wound edges. A delayed epithelialization can lead to superinfection and granulation formation, which further prevents epithelial healing. Those ears are most often treated on an outpatient basis by regular otomicroscopic suction cleaning and instillation of topical ear drops after removal or cautery of granulation tissue. This treatment has a high rate of controlling otorrhea; only resistant cases require additional modes of treatment.

Autologous split-thickness skin grafts have been most often used as they are readily available, eg, behind the ears, and give immediate coverage. However, they present major disadvantages: the thickness of the graft prevents good sound transmission, the graft removal requires a small intervention under local anesthesia, and above all, the grafts present a tendency to excessive desquamation. In addition, the loss of the unique property of self-cleansing by migration of the epithelial cells in the depth of the outer ear canal can lead to the accumulation of debris and enhances the risk for epidermoid cyst formation.

The use of cultured keratinocyte layers in the treatment of severe burn wounds and of chronic leg ulcers has been extensively investigated by several centers.[4-9] At the Brussels Burn Center,[4] large burn wounds are now routinely covered not only with autologous meshed split-thickness skin, but also with superimposed sheets of autologous or allogeneic cultured keratinocytes. The epithelialization of the interstices of the meshed skin was thereby found to be accelerated twofold as compared with wound beds covered only with meshed autograft. This twofold stimulation of epithelialization was documented with both autologous and allogeneic keratinocyte cultures.[4]

In 1991 and 1992 we treated ears presenting continuous and resistant otorrhea with autologous cultured skin transplants taken from the depth of the contralateral ear canal. However, culture proved successful in only 2 of the 11 selected patients. The constant fear of damaging the healthy ear often resulted in specimens too small for culture, and they were often contaminated. Also, the long waiting period (3 to 4 weeks) required for expansion of

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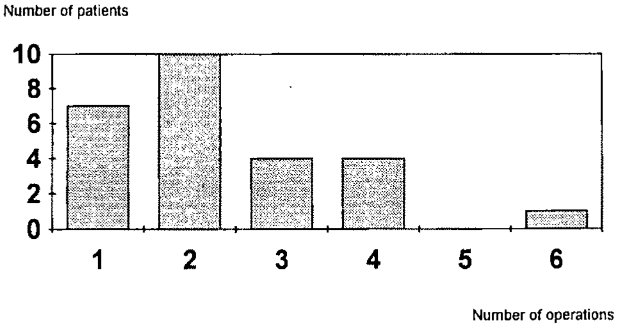

Number of operations undergone by patients prior to keratinocyte treatment.

keratinocytes to obtain multilayer keratinocyte sheets ruled out efficient treatment. In case the graft did not take, the procedure was also difficult to repeat. Since the results of burn wounds treated with allograft cultures were similar to those of wounds treated with autograft cultures, we decided in 1993 to switch to allogeneic keratinocyte cultures. For practical reasons also, allografts are preferred: no waiting period is required for culture, and the cryopreserved grafts are available at all times.

## PATIENTS AND METHODS

*Patient Selection and Population.* A total of 26 patients were admitted to the study. The age of the patients averaged 33.4 years, ranging from 7 to 60 years. All 26 patients had undergone previous surgery for cholesteatoma (n = 10), noncholesteatomatous otitis media (n = 8), or atresia (n = 8). The number of operations per patient varied from 1 to 6 (see Figure).

The technique used at the last surgical intervention to try to definitively solve the problem of otorrhea was either a closed one (20 cases) or an open one (6 cases). The mean duration between the onset of otorrhea after the last surgery and the new treatment modality averaged 37 months (range 3 months to 22 years). Fourteen patients had previously been followed for more than 1 year in our outpatient clinic, and many treatment modalities had been tried to stop the otorrhea and to improve the epithelialization.

To make sure the continuous otorrhea was not due to improper, unsatisfactory, or irregular treatment, we accepted only ears that for 3 months had been intensively treated by thorough suction cleaning, removal of granulation tissue, and application of trichloroacetic acid (15%) every 1 or 2 weeks according to the severity of the discharge. Daily instillation of antibiotic ear drops was carried out by the patients. Only ears that failed to heal after this intensive treatment were enrolled in the study and served as internal controls to be compared (after the additional 3 months of intensive therapy) with the same ears treated with use of keratinocyte sheets.

The authors excluded all ears in which the ear canal or mastoid cavity was not well isolated from the mesotympanum: tympanic perforations, uncontrollable retraction pockets, or active cholesteatomatous disease. Although the application of epidermal growth factor on perforations does not seem to induce cholesteatoma formation in animal studies,[10] we decided for safety reasons to exclude ears presenting a communication with the mesotympanum. We excluded also those ears in which a bony bare surface was present in the mastoid cavity or ear canal, because a vital wound bed is essential for epithelial overgrowth.

*In Vitro Culture of Keratinocytes.* Keratinocyte cultures were established from myringectomy specimens obtained from donor patients who needed a tympanoplasty procedure because of noncholesteatomatous chronic otitis media. At the time of tissue donation, blood was tested and was found negative for hepatitis B virus, hepatitis C virus, human immunodeficiency virus 1 and 2, syphilis, and cytomegalovirus. Since, in our department, tympanoplasty is usually performed with tympano-ossicular homografts, a total myringectomy is carried out with conservation of the epithelial lining in the depth of the ear canal.[3] The eardrum and the annulus were immersed in a transport medium containing penicillin G sodium and streptomycin sulfate and sent within 12 hours to the laboratory. Keratinocyte culturing was based on the technique of Rheinwald and Green.[11] The dermis was separated from the epidermis by incubation of the specimen in trypsin and collagenase in order to remove the fibrous elements of the eardrum. The obtained suspension of primary keratinocytes was centrifuged and the pellet was brought again into suspension with 10 mL of culturing medium. Those primary keratinocytes were seeded in a 25-cm$^2$ tissue culture flask on a feeder layer of 300,000 mitomycin C–treated Swiss 3T3-fibroblasts. The culture medium was changed every 2 days and the culture was observed daily for the appearance of keratinocyte clones. Fibroblasts were washed away with a solution of 0.2% trypsin (Life Technologie, Belgium) and 0.02% ethylenediaminetetraacetic acid (Sigma, NTL, Belgium) in phosphate-buffered saline. After confluence the cells can be picked up for serial subculture up to passage 4 (with a 1:3 split ratio at each passage). Of the 49 tympanic membranes brought in for culture, only in 3 cases could subculture be reached up to passage 3 (n = 2) or 4 (n = 1). In 34 cases the culture became contaminated. In 5 cases no growth was observed and in 6 cases the human fibroblasts overgrew the keratinocyte cells. A total of 48 passage 3 and 23 passage 4 sheets (each 25 cm$^2$) were produced

PATIENT DATA

| *Patient No.* | *Age (y)* | *No. of Operations* | *Indication for Surgery* | *Last Technique Used* | *Duration of Otorrhea (mo)* | *No. of Applications* | *Follow-up Duration (mo)* | *Result* |
|---|---|---|---|---|---|---|---|---|
| 1 | 7 | 1 | Atresia | C | 3 | 4 | 11 | ± |
| 2 | 33 | 4 | COM | C | 6 | 1 | 11 | + |
| 3 | 38 | 2 | COM | C | 60 | 4 | 22 | ± |
| 4 | 52 | 1 | COM | C | 6 | 1 | 8 | + |
| 5 | 25 | 1 | Atresia | C | 9 | 6 | 11 | – |
| 6 | 32 | 3 | Cholest | C | 12 | 4 | 11 | + |
| 7 | 47 | 4 | Cholest | C | 12 | 3 | 6 | + |
| 8 | 9 | 2 | Atresia | C | 48 | 2 | 10 | + |
| 9 | 34 | 2 | Cholest | C | 8 | 2 | 6 | + |
| 10 | 53 | 2 | Cholest | O | 72 | 11 | 14 | – |
| 11 | 16 | 2 | COM | C | 14 | 2 | 14 | + |
| 12 | 19 | 2 | Atresia | C | 9 | 2 | 12 | + |
| 13 | 32 | 1 | Atresia | C | 4 | 10 | 14 | ± |
| 14 | 38 | 6 | Atresia | O | 260 | 2 | 10 | – |
| 15 | 15 | 2 | Cholest | O | 9 | 1 | 12 | + |
| 16 | 36 | 3 | Cholest | O | 6 | 2 | 7 | + |
| 17 | 52 | 3 | Cholest | C | 21 | 2 | 11 | + |
| 18 | 58 | 4 | COM | C | 60 | 3 | 9 | + |
| 19 | 43 | 3 | COM | C | 132 | 2 | 12 | – |
| 20 | 60 | 2 | Cholest | O | 5 | 3 | 15 | + |
| 21 | 44 | 1 | COM | C | 4 | 2 | 6 | + |
| 22 | 21 | 2 | Atresia | C | 48 | 2 | 9 | + |
| 23 | 33 | 4 | Cholest | O | 84 | 3 | 5 | + |
| 24 | 9 | 1 | Atresia | C | 3 | 2 | 11 | + |
| 25 | 23 | 1 | COM | C | 38 | 5 | 12 | – |
| 26 | 40 | 2 | Cholest | O | 36 | 3 | 7 | + |

COM — chronic otitis media, Cholest — cholesteatoma, C — closed, O — open, ± — temporary success, + — success, – — failure.

with the 3 successful subcultures. Upon confluence of the final passage, cultures were allowed to stratify into multilayered sheets for an additional week, in order to allow easier manipulation of the keratinocyte sheets. The flask was opened by the use of a heating wire and the confluent keratinocyte layer was detached by means of 10 mL of Dispase (2.5 μg/mL, Boehringer Mannheim, Belgium). A carrier gauze (N-terface, Hospithera, Belgium) was put on top of the keratinocytes and the borders were folded back onto this plastic gauze. The culture could then be taken out of the tissue flask for storage in an aluminum bag and was processed through a cryopreservation program and stored in liquid nitrogen. This ensures permanent access to frozen sheets in the outpatient otolaryngology clinic. After thorough suction cleaning of the ear, the keratinocyte sheet was thawed and applied in one or several pieces into the depth of the ear canal or mastoid cavity. On an irregular mastoid surface, small pieces were cut and arranged as a patchwork to obtain a close contact with the surface to be covered. The patient was sent home with Bacicoline-B (Chibret) ear drops containing colistimethate sodium 20 mg/mL and bacitracin 8 mg/mL, which were found to be noncytotoxic for the keratinocyte cultures. One week later, the plastic carrier sheet was removed and a new keratinocyte layer was applied if healing was not complete. The ear was controlled every week and reapplication was performed until complete epithelialization and cessation of otorrhea were reached. Follow-up was thereafter secured by monthly assessment. The maximum number of applications was fixed at 6. Persisting otorrhea was considered a failure.

## RESULTS

A total of 84 applications were performed in our 26 patients (see Table). The average number of previous operations (mostly elsewhere) was 2.4. Eight ears had previously been operated on for chronic otitis media, 8 for atresia, and 10 for cholesteatomatous disease. The last technique used was a closed one in 19 cases and an open radical cavity in 7. Six of the 10 cholesteatomatous ears had been treated by the open technique. The duration of chronic otorrhea before the keratinocyte grafting averaged 37 months (range 3 months to 22 years). The average number of applications per patient was 3.2 (range 1 to 11). The average follow-up time was 9.8 months (range 6 to 22 months). Complete epithelialization and cessation of discharge was obtained in 18 of the 26 cases (69%). In those successful cases, an average of 2.2 applications proved necessary to dry the ear. In those ears progression of healing in the depth of the outer ear canal, eardrum, and/or mastoid cavity was observed by weekly otomicroscopic examination and was characterized by a progression of the epithelial front, with simultaneous regression of the exudating granulation area. The otorrhea most often disappeared after 2 applications, requiring in some cases a third one to obtain a totally closed and epithelialized surface. In the first weeks, neovascularization by capillary sprouting was often observed under the new epithelial layer and was gradually followed by a decrease in capillary proliferation and an increase in subepithelial (probably fibroblastic) organization.

A temporary epithelialization with repeated need for reapplication after a symptom-free period of 3 months was observed in 3 patients (12%). Absence of epithelialization with uninterrupted otorrhea meant failure: this was found in 5 patients (19%). An explanation for partial failure with temporary cessation of otorrhea could be found in 2 of the 3 cases. Case 1 was a bilateral atresia patient wearing, all day long, an air conducting hearing aid closing off the ear canal, thereby causing maceration and superinfection. Case 13, also an atresia patient, presented a too-narrow, almost stenotic, meatoplasty.

In 3 of the 5 cases a reason for total failure could be found. Case 15, with a large mastoid cavity, presented uninterrupted granulation formation, despite thorough, repeated granulation removal, cleaning, and several keratinocyte applications. During subsequent revision surgery, a large foreign body granuloma was found overlying a piece of the surgical packing left behind by the previous surgeon. Case 10, with an open cavity, presented residual mastoid cells with areas covered by respiratory mucosa (as proven by biopsy) causing recurrent discharge during upper respiratory tract infections. This patient is waiting for revision surgery. In case 23, a closed technique for chronic otitis media had been used, but after a trouble-free healing period the graft started to present a chronic granulomatous myringitis in the anterior half of the eardrum. After unsuccessful treatment including allograft sheets, the ear was reoperated and an edematous "sick" mucosa was found filling the whole tympanic cavity.. After thorough removal of this hyperplastic mucosa and myringoplasty with allograft tympanic material, a dry eardrum was finally achieved. In 2 of the 5 failed cases no reason for lack of epithelial healing could be disclosed.

## DISCUSSION AND CONCLUSION

The three subgroups in our study population are mastoid cavities, tympanoplasties, and operated atretic ears. All the studied ears had in common the absence of epithelial coverage in the depth of the outer ear canal or mastoid cavity. It has long been known that the epithelium of the ear canal presents the unique ability to migrate.[12-14] The absence of this self-cleaning mechanism and the permanent fluid exudation from uncovered areas leads to fluid stagnation and accumulation of debris, which form the ideal culture medium for bacteria. Once infection sets in, granulation tissue develops, which further impedes epithelial healing. By suction cleaning and instillation of antibiotic ear drops, this vicious circle can often be interrupted and spontaneous epithelial healing can take place. However, in some cases the epithelial repair mechanism seems to be defective or too slow.

The reported incidence of intermittent or persistent otorrhea following open cavity mastoidectomy varies from 20% to 60%.[1] This is one of the reasons why, in our department, we seldom perform an open technique for eradication of cholesteatoma, the open cavity technique being reserved for giant primary cholesteatoma or recurrent, rapidly extensive cholesteatoma.

All 6 discharging cavities presented a proper low facial ridge, a large meatus, and a middle ear isolated from the cavity. In 5 cases a dry cavity was obtained after an average of 2.4 applications. In 1 case, uninterrupted otorrhea compelled us to perform revision surgery, which revealed some of the surgical packing left behind by the previous surgeon. Cell grafting seems, therefore, to be a good treatment modality of last resort before revision tympanoplasty or mastoid surgery is undertaken.

Reconstructive surgery for atresia remains a much-debated subject — even more so with the advent of bone-anchored hearing devices.[15] We now operate only on cases with favorable anatomy as revealed by computed tomography scan, where after creation of a new ear canal and tympanic membrane a good hearing result can be expected. In those cases, a canalplasty with tympano-ossicular allografting is preferred because of the proven better long-term results as compared with the classic large cavity techniques, which often present a granulomatous covering of the cavity. But even with the allograft technique, when the extraconchal nonmigratory skin does not grow over the graft, the tympanic membrane and the depth of the newly created canal may be covered, in 14% of the cases by a granulomatous and often suppurative layer, as reported by Marquet and Declau.[2] In 4 of our 7 granulating ears operated on by the Marquet technique,[2] complete healing was reached. Two were temporarily dry; 1 continued to discharge.

In our experience, chronic granulomatous myringitis rarely complicates treatment of chronic otitis media by closed technique tympanoplasty: between 1% and 2% of cases.[3] After every myringotympanoplasty, a competition sets in between the natural tendency of a graft to necrosis and the epithelial coverage and fibrosis of the autologous or allogeneic scaffold material.[16] All factors delaying the natural epithelial coverage can lead to troublesome granulomatous myringitis. It is generally due to excessive removal of skin from the depth of the external auditory canal, to improper postoperative follow-up, or to premature postoperative contact with water, such as by swimming.

Although most cases studied here have been followed and treated for months or even years, only ears that were intensively treated in the outpatient department for another 3 additional months and failed to heal were brought into the study.

With regard to the long-standing and often crippling otorrhea that all 26 patients presented prior to grafting cultured keratinocytes, these allografts did provide a long-term epithelial repair in a high percentage of ears (69%). Similar results were obtained by Premachandra et al[17] using keratinocyte autografts. They reported in 17 of their 26 cases (65%) a complete epithelial healing without evidence of recurrence of granulation tissue after a follow-up period ranging from 10 to 18 months. All were mastoid cavities. In their study population, partial success with only temporary improvement in aural discharge was found in 7 cases (27%), whereas in 3 patients (8%) the discharge continued unabated after grafting. They used for culture a full-thickness retro-auricular autologous skin biopsy. This technique has the disadvantage that the original migratory epithelium is replaced by nonmigratory cells, which can prevent migration of surrounding migratory cells and in the long run lead to accumulation of debris. On the other hand, however, there is a real take and survival of autologous cells, which is not the case for allogeneic cells.

Up to now, tympanic and annular keratinocyte culture has been mainly used as a research tool in order to compare the normal in vitro keratinocyte migration pattern with that of cholesteatomatous keratinocytes.[18] In this study, cultured "ear" keratinocytes were used for the first time for therapeutic means.

The observed enhancement of epithelial coverage by these cultured allograft keratinocytes is not due to a permanent take of the grafts, as shown by several studies on burns and chronic ulcers, but has generally been attributed to an indirect stimulation of the recipient's repair mechanisms.[4-9,19,20] Several groups have shown that in burn wounds the survival time of these allocultured grafts is limited, and that they are gradually replaced by autologous cells.[19,21] Initially, some authors claimed that these cultured allografted cells were not rejected, because Langerhans cells, regarded as critical in the process of skin graft rejection, are lost after 7 or more days in keratinocyte cultures.[19] More recent studies using human leukocyte antigen analysis,[22] Y chromosome analysis[21] in sex-mismatched grafts, and DNA fingerprinting techniques[19] have shown that host keratinocytes replace the allograft. This suggests that these viable allografts do not remain in situ permanently. Therefore, the observed wound repair–promoting effects are explained not by a grafting process per se, but by the stimulation of the endogenous wound repair mechanisms by the allografted cells. The release of growth factors during autolysis of the cells probably induces wound closure by stimulating the migration and proliferation of resident epithelial cells present at the wound edges.

Indeed, numerous growth factors and cytokines have been reported to be synthesized by epidermal cells. These include TGF-α, a-FGF, b-FGF, PDGF, TGF-β, amphiregulin, HB-EGF, NGF, TNF-α, IL-1α, IL-1β, IL-6, IL-8, GM-CSF, G-CSF, and M-CSF.[4,10,18-20,23-25] Many of these factors have important effects on one or more stages of the wound healing process. TGF-β, the interleukins, and the colony stimulating factors are powerful modulators of the postwounding immune response. Angiogenesis is stimulated by the fibroblast growth factors. Fibroblast proliferation is promoted by PDGF, FGFs, and EGF-like factors, and the deposition of extracellular matrix is promoted by TGF-β.[4] Both FGFs and EGF-like growth factors are important keratinocyte mitogens, and may promote wound closure by stimulation of keratinocyte proliferation and migration.[25] Several animal studies have shown the effect of these two keratinocyte stimulating factors on acute or chronic tympanic membrane perforations. Animal studies by Lee et al[10] with recombinant epidermal growth factor (EGF) showed a significantly higher rate of closure of induced chronic perforations as compared to controls. Also, those EGF-healed tympanic membranes were found to be histologically similar to normal tympanic membranes, as opposed to the few spontaneously healed tympanic membranes from the control groups, where the thickness was only half that of normal tympanic membranes.

In acute perforation in rats, Mondain and Ryan[23] also showed similar accelerated healing, but with basic fibroblast growth factor (b-FGF). The histologic results of these healed eardrums demonstrated that b-FGF can produce a tympanic membrane scar containing more connective tissue, which may be of benefit in the prevention of atrophic tympanic membranes. Histologic studies of neomembranes in human eardrums by Govaerts et al[26] have clearly shown that the lamina propria can be atrophic, with fibroblasts absent, and with disoriented fibers.

In conclusion, our study confirms some of the current views on the biologic mechanism by which allocultured keratinocytes, despite their short postgrafting survival time, are able to promote the wound healing process, most probably by the release of growth factors. It is, however, still unclear which growth factors are involved, although we have detected b-FGF in keratinocyte extracts (unpublished results).

What is the level of activity of these cultured keratinocyte sheets compared with growth factors? Recently, Duinslaeger et al[4] showed that the total amount of mitogenic activity in the keratinocyte cell extract from a 175-cm$^2$ culture is equivalent to the activity produced by 40 to 80 μg of EGF. This implies that the amount of activity used in the present study (about 2 cm$^2$ per application) is very low as compared with the microgram amounts usually used in in vivo studies. This may support the growing awareness that growth factor therapy will probably only be maximally effective if multiple factors, which are only marginally effective upon isolated use, are combined. Since the different factors may have different activities or target cell specificities, such combinations may be able to modulate the different stages of the healing process more effectively than any single factor.

The future will tell us which of the options contains the greatest therapeutic value: the bulk administration of all intracellular components (as with cultured cells), or the selective, but possibly marginally effective, application of a single growth factor.

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ACKNOWLEDGMENT — We thank Dr Alain Vanderkelen, Head of the Burn Center, Military Hospital, Brussels, for encouraging the collaboration between the two centers, and for his advice.

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## THIRTY-SECOND POSTGRADUATE COURSE IN EAR SURGERY

The Thirty-Second Postgraduate Course in Ear Surgery will be held in Nijmegen, the Netherlands, from March 31 to April 5, 1997. For information, contact Prof P. van den Broek, University Hospital Nijmegen, Department of Otorhinolaryngology, PO Box 9101, 6500 HB Nijmegen, the Netherlands; fax 024-3540251.

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## INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

The biannual meeting of the Interamerican Association of Pediatric Otorhinolaryngology will be held in Sao Paulo, Brazil, September 21-24, 1997. For information, contact Office of the Secretary, Tania Sih, MD, PhD, Rua Itapeva, 366, Suite 102, 01332-000, Sao Paulo SP, Brazil; telephone 55 11 283-4645 or 283-3396; fax 55 11 826-9652 or 542-6037.

P24746

*Ann Otol Rhinol Laryngol* 106:1997

# SUDDEN DEAFNESS: A COMPARISON OF ANTICOAGULANT THERAPY AND CARBOGEN INHALATION THERAPY

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Sudden deafness (SD) is a sudden or rapidly progressive, partial or complete, typically unilateral sensorineural hearing impairment that has no known specific etiologic factor. This study was designed to compare, in a group of 168 consecutive patients with SD, the effect of anticoagulant therapy and carbogen inhalation therapy. Special attention was paid to the shape of the audiogram relative to the clinical outcome and the treatment modality. We found that the configuration of the audiogram of SD patients is prognostic of the outcome, and that patients with a low-frequency–sloping hearing impairment have a better prognosis compared to the patients with a high-sloping loss. Anticoagulant treatment was most effective in low-sloping hearing losses, while carbogen inhalation may be more effective for patients with high-sloping hearing losses.

KEY WORDS — anticoagulant therapy, carbogen inhalation therapy, sudden deafness.

## INTRODUCTION

Sudden deafness (SD) is a sudden or rapidly progressive, partial or complete, typically unilateral sensorineural hearing impairment that has no known specific etiologic factor. Insufficient knowledge of the pathogenesis and the lack of individual audiologic data preceding the SD, in addition to a high spontaneous recovery rate, complicate the study of sensorineural hearing loss and investigation of different treatment modalities.

Etiologic studies have led to four main theories for SD: circulatory disturbances (thrombosis, vasospasm), viral infection, membrane rupture, and autoimmune processes. Each of these four main theories has some support in the literature: Belal[1] described in 1980 changes in the cochlea after a labyrinthine artery occlusion and even in temporal bones of 2 patients with SD. He concludes that an occlusive arterial disease might play a role in some sudden sensorineural hearing losses.

The treatment modalities are mostly based on improving the blood circulation or oxygenation of tissues of the inner ear. Corticosteroids are also widely used in order to reduce presumed tissue swelling associated with viral infection or as a treatment for presumed autoimmune disease.

Simmons[2] describes 15 patients with a history of SD whose exact time of onset is known. He suggests that these cases are due to a rupture in the membranous cochlea.

Takayuki et al[3] conclude in their study that improving the blood circulation is important also for those cases of SD that are due to viral infections, because the viral infection itself also causes a decrease in the circulation due to viremia and a formation of edema at the intima of the inner ear blood vessels. In their study comparing anticoagulant therapy to betamethasone therapy, the results were better in the former group.

On the other hand, Wilkins et al[4] concluded in their study on 109 patients that treatment including dextran, histamine, Hypaque, diuretics, steroids, vasodilators, and carbogen inhalation gave results no better than those reported for spontaneous recovery.

Sheehy[5] in 1960 proposed that vasodilator therapy should always be started immediately in SD patients. As favorable prognostic signs he mentions low-frequency–sloping audiograms, hearing loss to 45 dB or less in speech frequencies, and the absence of vertigo.

Fisch et al,[6] as early as 1976, described increased oxygen tension in human perilymph caused by inhalation of a carbogen gas mixture. In a 1983 study, Fisch[7] recommended carbogen inhalation as an effective, noninvasive treatment for SD.

It seems evident that different types of sudden inner ear disorders may underlie SD. A major difference may be between pure cochlear and mixed vestibulocochlear disorders. The involvement of the vestibular organ may have prognostic value.[8,9]

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TABLE 1. BLOOD TESTS PERFORMED IN SUDDEN DEAFNESS PATIENTS IN ADDITION TO DAILY PURE TONE AND SPEECH AUDIOGRAMS

| |
|---|
| Total blood count |
| Sedimentation rate |
| Thrombocytes |
| Cholesterol (high-density lipoprotein and low-density lipoprotein) |
| Triglyceride |
| Fasting blood sugar level |
| Creatinine |
| Activated partial thromboplastin time |
| Partial thromboplastin time |
| Glutamyl transferase |
| Electrolytes (sodium and potassium) |

The shape of the audiogram curve also seems to have prognostic value: a good prognosis is associated with a flat, "cookie-bite," or upward-sloping audiogram, while a downward-sloping audiogram or profound hearing loss is associated with a poorer outcome.[10]

This study was designed to compare the effect of anticoagulant therapy and carbogen inhalation therapy in the treatment of SD patients. Special attention was paid to the correlation of the shape of the audiogram with the observed clinical outcome and the treatment modality applied.

## MATERIALS AND METHODS

*Patients.* One hundred sixty-eight patients suffering from SD were analyzed in this study. All patients had been treated in the Department of Otorhinolaryngology at Turku University Central Hospital in the period 1982 to 1989. The material includes 91 males and 77 females with a mean age of 51 years, ranging from 12 to 78 years. The patients were admitted to the hospital for acute sudden hearing loss, and the treatment was started promptly after the diagnosis of SD.

*Treatment Modalities.* The patients were divided into three different treatment groups: group 1 was treated with anticoagulant therapy, group 2 received both anticoagulant therapy and carbogen (5% oxygen and 95% carbon dioxide) inhalation therapy, and group 3 was treated only with carbogen inhalation therapy.

The anticoagulant therapy included intravenous heparin sodium 2 mL, 4 times daily for 2 days, and oral warfarin sodium at a dose sufficient to yield a thromboplastin time of about 0.10 (0.05 to 0.15), for 30 to 90 days. These patients also received oral betahistine hydrochloride, a vasodilative agent with effects shown on the cochlear blood flow in animals,[11] 8 mg 3 times a day, for the same period.

TABLE 2. DIVISION OF SUDDEN DEAFNESS PATIENTS ACCORDING TO TREATMENT MODALITY AND AUDIOMETRIC CONFIGURATION

| *Treatment Group* | *Type of Hearing Loss* Low | *Flat* | *High* | *Total* |
|---|---|---|---|---|
| 1 | 13 | 31 | 12 | 56 |
| 2 | 16 | 27 | 13 | 56 |
| 3 | 12 | 29 | 15 | 56 |
| Total | 41 | 87 | 40 | 168 |

The carbogen inhalation therapy required inhalation of the gas mixture, with a facial mask, 6 to 8 times daily for periods of 20 to 30 minutes over 4 days. The oral betahistine hydrochloride was also started for most of the patients. During this period subjects were inpatients at the Department of Otorhinolaryngology and all the tests listed in Table 1 were performed. Repeat evaluation was performed after 1 month and again, if the patient had not fully recovered, 3 months later. Pure tone audiograms were taken every day during hospitalization and during the subsequent retest visits.

## RESULTS

We classified SD patients into three groups according to the shape of their audiograms:

TABLE 3. HEARING IMPROVEMENT AFTER THREE MONTHS OF TREATMENT IN PATIENTS WITH SUDDEN DEAFNESS

| *Group* | *Hz* | *Low* | *Flat* | *High* |
|---|---|---|---|---|
| 1 | 500 | 41.9 | 15.0 | 12.2 |
| | 1,000 | 40.7 | 17.0 | 14.8 |
| | 2,000 | 30.4 | 14.9 | 8.4 |
| | 4,000 | 19.0 | 5.1 | 5.1 |
| | 6,000 | 14.9 | 5.3 | 9.4 |
| | 8,000 | 9.2 | 4.7 | 4.0 |
| 2 | 500 | 33.8 | 25.0 | 12.8 |
| | 1,000 | 27.1 | 22.7 | 13.6 |
| | 2,000 | 18.7 | 19.3 | 10.8 |
| | 4,000 | 13.6 | 17.1 | 5.6 |
| | 6,000 | 11.3 | 10.3 | 7.3 |
| | 8,000 | 11.4 | 12.5 | 6.5 |
| 3 | 500 | 24.5 | 31.8 | 22.7 |
| | 1,000 | 24.1 | 30.7 | 23.0 |
| | 2,000 | 16.8 | 20.0 | 17.7 |
| | 4,000 | 19.0 | 12.6 | 13.6 |
| | 6,000 | 8.2 | 11.3 | 5.6 |
| | 8,000 | 11.8 | 10.4 | 6.1 |
| 1 | Mean | 37.8 | 16.5 | 11.4 |
| 2 | Mean | 26.2 | 22.1 | 11.8 |
| 3 | Mean | 21.1 | 27.3 | 21.3 |

Data are in decibels.

Group 1 — anticoagulant treatment; group 2 — anticoagulant plus carbogen; group 3 — carbogen; low — low-sloping audiogram; flat — flat or variable audiogram; high — high-sloping audiogram; mean — mean of 500-, 1,000-, and 2,000-Hz values.

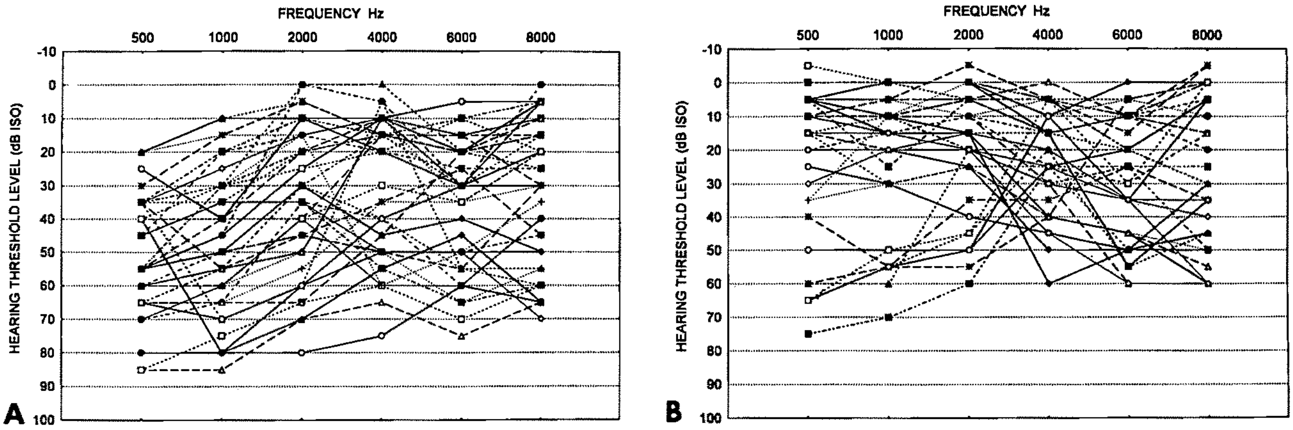

Fig 1. Individual audiograms at A) time of diagnosis and B) at control at 3 months for patients with hearing loss predominantly in low frequencies and classified as "low-sloping audiograms."

1. Patients with low-frequency (upward-sloping) hearing loss ("low").
2. Patients with flat or variable audiograms ("flat").
3. Patients with high-frequency (downward-sloping) hearing loss ("high").

Group 1 (low) includes the patients whose pure tone thresholds at low frequencies (125, 250 Hz) were at least 15 dB below the mean threshold demonstrated for the speech frequencies (500, 1,000, 2,000 Hz) at the time of diagnosis. Group 3 (high) includes the patients whose high-frequency (3,000, 4,000, 6,000, and 8,000 Hz) thresholds were at least 15 dB below the mean speech-frequency area at the time of diagnosis. The audiograms in this group were also matched according to age and sex by means of ISO standards (ISO-94; International Organization for Standardization, Geneva, Switzerland, 1994). Thus, "normal" matched impairment was accepted in patients 50, 60, or 70 years old. Patients with total or near-total losses (n = 25), U-shaped losses, or both low- and high-tone–sloped audiograms were classified together into the group of flat hearing loss. Those patients were randomly assigned to one of the three treatment modalities. Thus, nine groups existed, as presented in Table 2.

The three treatment groups were statistically comparable according to sex and age. The mean ages in groups 1, 2, and 3 were 52.3, 51.7, and 50.0, respectively. Table 3 shows the differences of the pure tone thresholds at the second control evaluation (3 months) compared to the time of diagnosis. If the second control was not available, results from the first control (1 month) were used. Figures 1-3 show the individual auditory threshold curves at the time of diagnosis (A) and at the second (if not available, the first) control (B) for each of the three audiometric categories: low (G1), flat (G2), and high (G3). Figure 1A (low) indicates that the values of the 0.5-Hz frequency are scattered between 20 and 85 dB, while in Fig 1B they are between –5 and 75 dB. Thus, there are remarkable changes, while the changes at the higher frequencies are clearly smaller. In Fig 2 the changes between A and B are not so obvious. The values are spread widely over all frequencies at the time of diagnosis. This reflects the fact that this

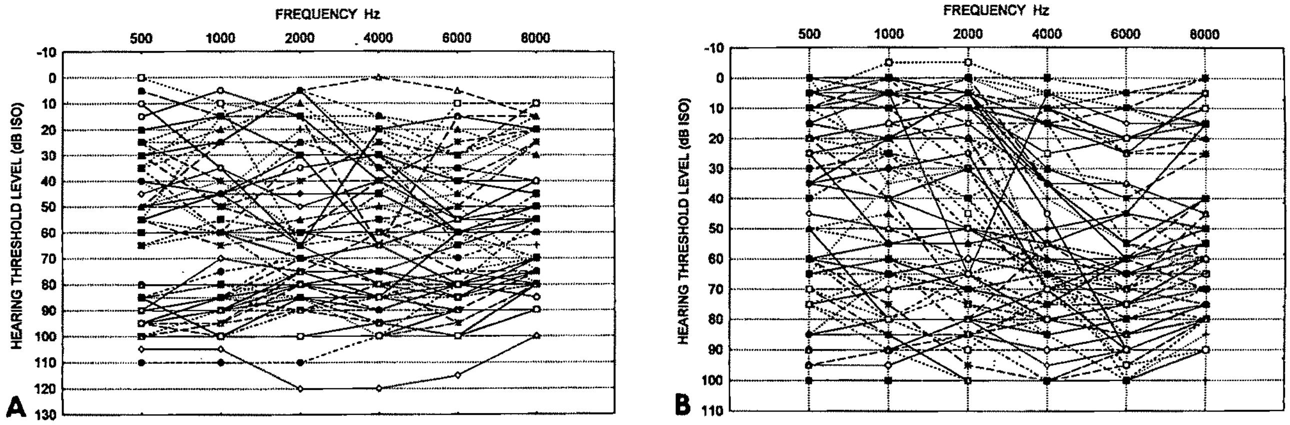

Fig 2. Individual audiograms at A) time of diagnosis and B) at control at 3 months for patients with flat, U-shaped, or both high- and low-tone–sloping audiograms, or near-total to total hearing loss and classified as "flat audiograms."

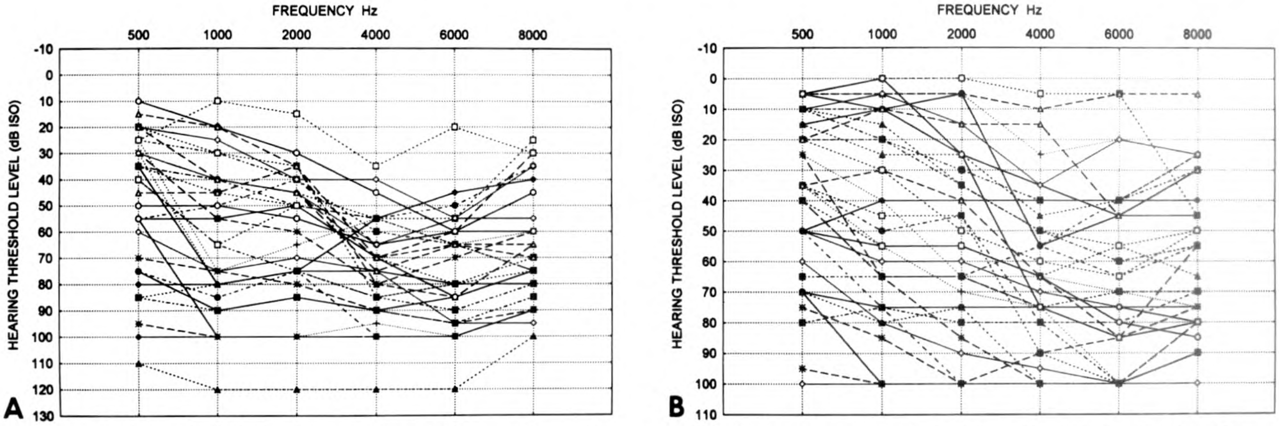

Fig 3. Individual audiograms at A) time of diagnosis and B) at control at 3 months for patients with hearing loss predominantly in high frequencies and classified as "high-sloping audiograms."

"flat"-audiogram group includes the U-shaped, both high- and low-tone–sloping, and total or near-total hearing loss audiograms. In Fig 3 (high) the frequencies 4, 6, and 8 kHz show most improvement at control (B) compared to the values observed at the time of diagnosis. There are some remarkable improvements, but the general shapes in high-sloping audiograms remain after treatment. Figure 4 presents the mean hearing threshold values (decibels) at 500, 1,000, and 2,000 Hz in treatment groups 1, 2, and 3 at the time of diagnosis, at 1 to 4 days following diagnosis, and at 1 and 3 months following diagnosis. The anticoagulant treatment group (Fig 4A) demonstrates hearing improvement in all these speech-area frequencies through the 1-month control test. However, at the 3-month control, the average hearing threshold levels are slightly poorer. The greatest improvement seems to happen between 1 to 4 days and the 1-month control. Figure 4B presents the anticoagulant plus carbogen therapy group. The hearing threshold levels at the time of diagnosis are better in this group than in treatment group 1, but the levels at the control evaluation are parallel to those of group 1. In this group the results are best at 1-month control measurements. Figure 4C presents the carbogen treatment group. In this group the hearing threshold levels seem to remain similar at 1- and 3-month control measurements at the frequencies 500 to 2,000 Hz. Note in Fig 4C that

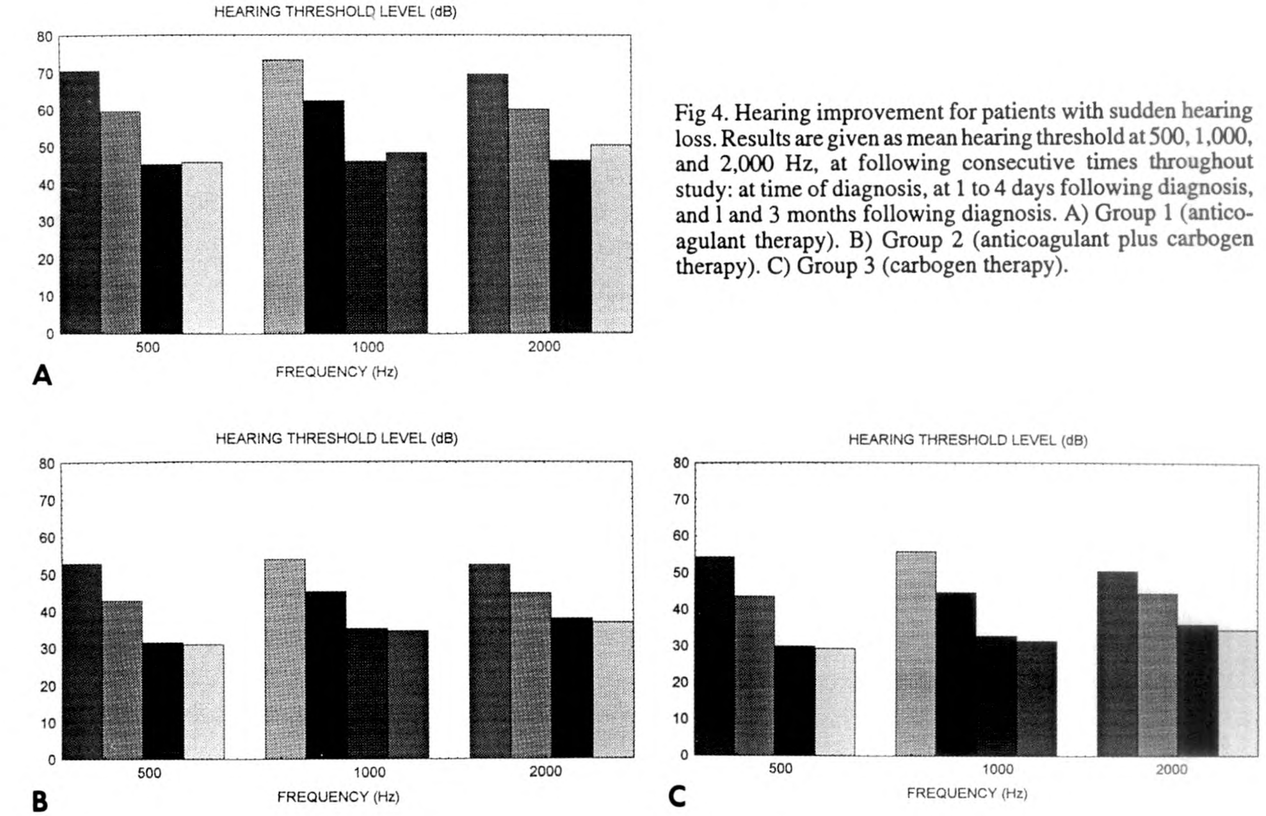

Fig 4. Hearing improvement for patients with sudden hearing loss. Results are given as mean hearing threshold at 500, 1,000, and 2,000 Hz, at following consecutive times throughout study: at time of diagnosis, at 1 to 4 days following diagnosis, and 1 and 3 months following diagnosis. A) Group 1 (anticoagulant therapy). B) Group 2 (anticoagulant plus carbogen therapy). C) Group 3 (carbogen therapy).

the hearing continues to improve at these frequencies even after the measurement at 1 to 4 days.

## DISCUSSION

The hearing losses in our material were classified as flat in 52% of cases studied, and sloping to the high tones and to the low tones in 24% each. This result is consistent with Sheehy's.[5] He classified 223 SD patients into four groups: low-tone, flat, high-tone, and total loss of hearing. A flat type occurred in 41%, high type in 29%, low in 17%, and a total loss of hearing in 13% of the patients. Statistically there is no difference in the sexes between the three treatment groups in our material, nor is there a statistically significant age difference between the three treatment groups. The patients had only few known illnesses or medications. Blood test results showed no deviation from the results in a normal population. In considering the mean values of 500, 1,000, and 2,000 Hz, the improvement of hearing was best in group 1 among the patients with a low-sloped audiogram (37.8 dB). Similarly, in group 2, the improvement was 26.2 dB in the low-type hearing loss. In group 3 the flat type of audiogram had the best recovery (27.3 dB). It can be noticed that the patients with the high-frequency hearing impairments had the poorest prognosis in all treatment groups (Table 3). This result parallels results described in earlier literature.[10] It is, however, to be supposed that these patients with high-frequency–sloping audiograms often had hearing changes preceding their SD, eg, reflecting age-related hearing loss, while the patients with low-sloping hearing impairment more often had normal audiograms before the SD. Table 3 shows the hearing improvement results in the different groups for individual frequencies from 500 to 8,000 Hz.

Anticoagulant treatment (group 1) seems to yield best hearing threshold improvement in low types of hearing losses, while carbogen (group 3) gives better results in flat and high types of hearing losses. The combination treatment seems to be truly effective only for 4,000-Hz and 8,000-Hz tones.

Thus, we conclude that the configuration of the audiogram of SD patients has a prognostic value and that the patients with low-frequency–sloping hearing impairment have a better prognosis than patients with high-frequency–sloping losses. Anticoagulant treatment seems to be more effective in low-sloping hearing losses, while carbogen inhalation can be recommended in high-sloping hearing losses.

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*Ann Otol Rhinol Laryngol* 106:1997

# BLOOD FLOW IN COMMON CAROTID AND VERTEBRAL ARTERIES IN PATIENTS WITH SUDDEN DEAFNESS

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Blood flow was measured in the common carotid artery (CCA) and the vertebral artery (VA) by the ultrasonic Doppler method in 14 male patients with sudden deafness and 70 normal adults. In the patients, blood flow on the affected side was slower than that on the normal side or that of the control group. Although these differences were not statistically significant in the CCA or in the VA, significant differences in the blood flow were noted between the group with a hearing loss of greater than 50 dB and the group with a loss of less than 50 dB. A negative correlation was found between blood viscosity and blood flow in both CCAs and both VAs. After stellate ganglion block (SGB), the blood flow of the CCA and VA increased on the side of the SGB and decreased on the opposite side. The changes in blood flow after SGB decreased with age, presumably because of changes in the blood vessel walls and a weaker response to sympathetic nerve receptors in the arterial wall.

KEY WORDS — blood flow, common carotid artery, Doppler ultrasound, sudden deafness, vertebral artery.

## INTRODUCTION

Treatments for sudden deafness (SD) have been aimed at improving cochlear circulation. Stellate ganglion block (SGB) can be used for this purpose together with other agents. However, controversy remains about the distribution of the sympathetic nerves that affect the blood vessels in the cochlea. Only a few reports are available of studies of the changes in the hemodynamics of vertebral arteries (VAs) after SGB. Using an ultrasonic color-coded Doppler method, we measured the blood velocity (V), blood flow (F), and cross-sectional area (A) of the common carotid artery (CCA) and VA in patients with SD before and after SGB and compared the results with those of controls.

## SUBJECTS AND METHODS

We studied 14 male patients with SD who underwent SGB and 70 healthy adults (35 males and 35 females); the age of the patients ranged from 26 to 65 years, and that of the controls from 25 to 68 years. Table 1 shows the age, affected side, average hearing

TABLE 1. AGE, SIDE AFFECTED, TYPE OF HEARING IMPAIRMENT, AVERAGE HEARING LEVELS, AND DURATION OF IMPROVEMENT

| *Age* | *Side* | *Pretreatment Audiogram Pattern* | *Average Hearing Level* (dB)* | | *Duration of Improvement (d)* |
|---|---|---|---|---|---|
| | | | *Pretreatment* | *Posttreatment* | |
| 26 | L | High-tone | 90 | 54 | 21 |
| 29 | L | Flat | 40 | 26 | 14 |
| 34 | R | Low-tone | 35 | 11 | 18 |
| 35 | L | Low-tone | 42 | 20 | 14 |
| 40 | L | Flat | 52 | 34 | 21 |
| 46 | L | Low-tone | 40 | 18 | 16 |
| 48 | R | Flat | 64 | 10 | 7 |
| 48 | L | Flat | 70 | 38 | 25 |
| 49 | L | Low-tone | 24 | 8 | 14 |
| 50 | L | Low-tone | 31 | 15 | 18 |
| 52 | R | Total deafness | 110 | 110 | 0 |
| 57 | R | Total deafness | 105 | 34 | 24 |
| 58 | L | Flat | 39 | 10 | 15 |
| 65 | L | Flat | 53 | 29 | 14 |

*Averaged for five frequencies: 250, 500, 1,000, 2,000, and 4,000 Hz.

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TABLE 2. BLOOD VELOCITY, BLOOD FLOW, AND AREA OF CROSS SECTION OF COMMON CAROTID ARTERY AND VERTEBRAL ARTERY IN CONTROLS

| | | *Carotid Artery* | | | *Vertebral Artery* | | |
|---|---|---|---|---|---|---|---|
| | | *Men (n = 35)* | | *Women (n = 35)* | *Men (n = 35)* | | *Women (n = 35)* |
| Blood velocity (cm/s) | R | 32.2 ± 11.8 | | 35.6 ± 10.6 | 29.7 ± 7.60 | ** | 37.1 ± 11.8 |
| | L | 32.9 ± 8.60 | ** | 39.8 ± 9.89 | 32.9 ± 8.36 | ** | 39.8 ± 9.89 |
| Blood flow (mL/s) | R | 10.2 ± 2.88 | | 9.98 ± 2.03 | 2.04 ± 0.93 | | 1.94 ± 0.91 |
| | L | 10.1 ± 3.04 | | 10.2 ± 3.02 | 2.19 ± 1.04 | | 2.33 ± 0.78 |
| Area of cross section (mm²) | R | 32.9 ± 7.63 | * | 28.5 ± 7.05 | 11.3 ± 3.37 | ** | 8.50 ± 2.39 |
| | L | 32.2 ± 8.51 | * | 27.7 ± 6.06 | 11.4 ± 3.55 | * | 9.56 ± 2.55 |

Data are mean ± standard deviation.
*p < .05.
**p < .01.

level, type of hearing impairment, and duration of improvement.

We employed an ultrasonic color-coded Doppler method, using Toshiba SSA-270A for ultrasonography and Toshiba PLF-730ST (7.5-MHz frequency, 1-mm distance resolution, and 2-mm azimuth resolution) as a probe. Measurements were carried out with a Doppler and color filter of 50 to 400 Hz at a pulse rate of 3 to 6 kHz, pulse width of 1 to 10 milliseconds, sample width of 1 to 10 mm, and detectable minimum V of 2 cm/s.

In the anterolateral part of the neck, 2 to 4 cm below the carotid bifurcation, the V, F, and A of the CCA were measured, and between the C-3 and C-5 vertebrae, those of the VA.

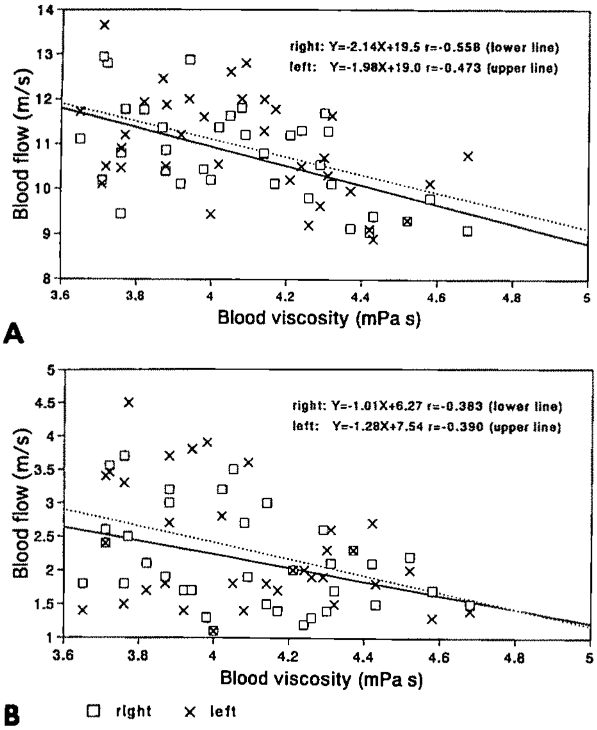

Fig 1. Relationship, in male controls, between blood viscosity and blood flow in A) common carotid artery and B) vertebral artery.

The SGB was performed by a paratracheal injection of 6 mL of 1% lidocaine hydrochloride at the base of the transverse process of C-7. Each parameter in the CCA and the VA was measured 10 minutes after SGB only in those with Horner's sign after SGB: blepharoptosis, miosis, and conjunctival hyperemia.

Blood viscosity was measured at a shear rate of 376.12 $s^{-1}$ with an E-type viscosimeter (Tokyo Instruments).

The treatment consisted of intravenous and oral medication and SGB for 16 days. The intravenous electrolyte solution contained 1 mg of vitamin $B_{12}$ (Methycobal, Eisai Co Ltd, Tokyo, Japan), 120 mg of adenosine triphosphate (Trinosin, Yamanouchi Pharmaceutical Co Ltd, Tokyo), and 10 µg of prostaglandin $E_1$ (Liple, The Green Cross Corporation, Osaka, Japan). Betamethasone disodium phosphate (Rinderon, Shionogi & Co Ltd, Osaka) was also administered as follows: 4 mg for the first 4 days, 3 mg for the second 4 days, 2 mg for the third 4 days, and 1 mg for the last 4 days. Oral medication of 150 IU of kallidinogenase (Carnaculin, Sanwa Kagaku Co Ltd, Nagoya, Japan) and 300 mg of pentoxifylline (Trental, Hoechst, Frankfurt, Germany) was given. The SGB was performed daily on the affected side Monday through Saturday.

## RESULTS

Table 2 shows the values for V, F, and A in the CCA and the VA of 70 healthy adults. Although there was no significant difference (by *t*-test) between the right and left CCAs and VAs, the V and F of the left CCA and VA were greater than those on the right, except for the F in the CCA of males. A comparison of male and female values by *t*-test revealed significant differences in V and A, but not in F. Figure 1 shows the relationship between blood viscosity and F in the CCA and VA of male controls. On both the right and left sides, negative correlations were found.

TABLE 3. BLOOD FLOW OF COMMON CAROTID ARTERY AND VERTEBRAL ARTERY IN PATIENTS WITH SUDDEN DEAFNESS AND CONTROLS

| *Blood Flow (m/s)* | | *Sudden Deafness (n = 14)* | *Controls (n = 14)* |
|---|---|---|---|
| Carotid artery | A | 9.90 ± 3.64 | 10.7 ± 3.24 |
| | O | 10.2 ± 3.43 | |
| Vertebral artery | A | 2.47 ± 1.23 | 2.61 ± 1.34 |
| | O | 2.89 ± 1.07 | |

Data are mean ± standard deviation.
A — affected side, O — opposite side.

Table 3 shows the pretreatment F of 14 patients with SD and 14 age-matched controls. Although F was slightly lower on the affected side than on the opposite side or in the controls, the difference was not significant (by *t*-test).

Figure 2 shows the pretreatment F of patients with SD. The data were correlated with average hearing level at the first medical examination (group A less than 50 dB; group B 50 dB or more). A comparison of F in the affected side between group A and group B by *t*-test revealed significant differences in CCA and VA.

Figure 3 shows the relationship between blood viscosity and F in the CCA and VA. On both the affected and opposite sides, negative correlations were found. There is no significant difference in regression line between right or left CCAs of the controls and the affected CCAs of patients with SD. In 22 of the 23 measurements in the CCA and in 14 of the 23 measurements in the VA, F was lower on the affected side. Since the incidence of SD was higher on the left side (10 left ears versus 4 right ears), the left CCA and VA appeared to be more susceptible to disturbances of blood flow.

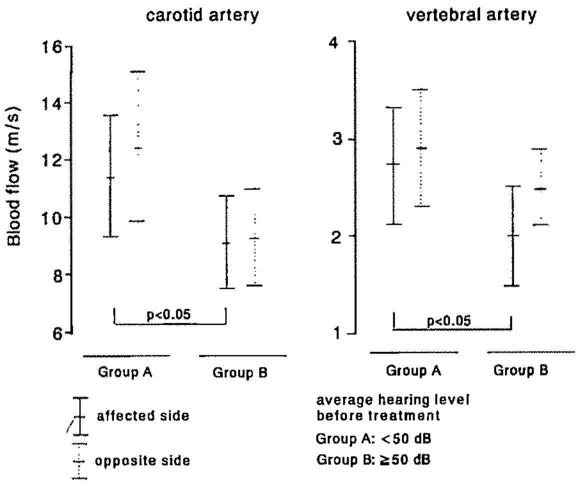

Fig 2. Blood flow of patients with sudden deafness (SD). In group A, average hearing level before treatment is less than 50 dB; in group B, 50 dB or more.

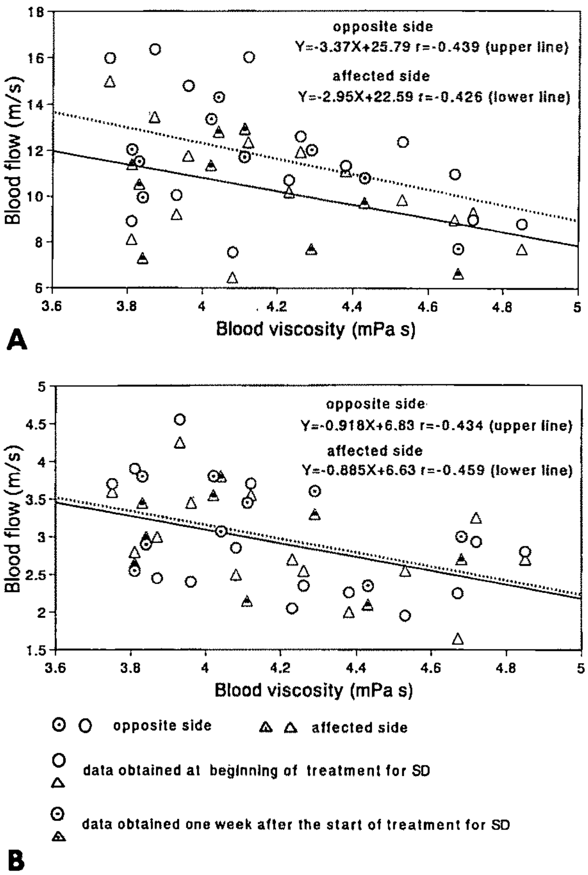

Fig 3. Relationship, in patients with SD, between blood viscosity and blood flow in A) common carotid artery and B) vertebral artery.

Figure 4 illustrates the change of F during treatment in 9 patients. In 6 of them, F in the CCA on the affected side was greater 1 week after the start of treatment than before treatment. On the opposite side, however, in 6 of the 9 patients, F was decreased 1 week after the start of treatment. In the VA, F was increased 1 week after the start of treatment in 5 of the 9 patients on both the affected and opposite sides.

The changes in each parameter after SGB are shown in Table 4. In the CCA, V and F increased on the affected side and decreased on the opposite side, and in the VA, V and F increased on the affected side but did not change on the opposite side. As demonstrated in Fig 5, the changes in percentage of 23 measurements after SGB showed a negative correlation with age in both the CCA and the VA on the affected side.

## DISCUSSION

We studied the hemodynamics of CCA and VA using the ultrasonic color-coded Doppler method, which displays two-dimensional color flow images and the pulse Doppler mode simultaneously, so a blood vessel with even a relatively small diameter can be identified easily by a color signal. Moreover,

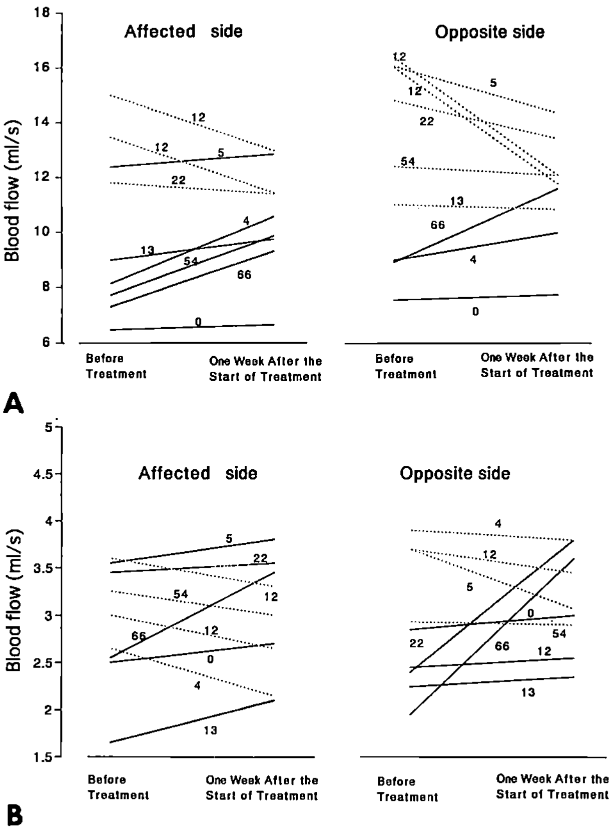

Fig 4. Changes of blood flow after start of treatment in A) common carotid artery and B) vertebral artery. On line connecting "Before Treatment" and "One Week after the Start of Treatment," hearing gain is expressed in decibels.

the angle made by the ultrasonic beam and the blood flow direction can be measured to give a more accurate quantitative assessment of the parameters of blood flow than that obtained by a simple ultrasonic Doppler method. Such accuracy in the values obtained by the above method is demonstrated by the data recorded in the present study (Table 2), which coincide closely with the results reported previously.[1-3]

TABLE 4. CHANGE OF BLOOD VELOCITY, BLOOD FLOW, AND AREA OF CROSS SECTION OF COMMON CAROTID ARTERY AND VERTEBRAL ARTERY AFTER STELLATE GANGLION BLOCK

| | | *Carotid Artery (n = 23)* | *Vertebral Artery (n = 23)* |
|---|---|---|---|
| Blood velocity (%) | A | 135.1 ± 23.2 | 129.0 ± 15.8 |
| | O | 89.7 ± 14.1 | 101.9 ± 14.8 |
| Blood flow (%) | A | 134.3 ± 18.1 | 130.5 ± 14.1 |
| | O | 87.1 ± 11.9 | 102.9 ± 11.2 |
| Area of cross section (%) | A | 113.1 ± 13.4 | 116.3 ± 14.0 |
| | O | 103.3 ± 9.42 | 98.92 ± 12.4 |

Data are mean ± standard deviation.
A — affected side, O — opposite side.

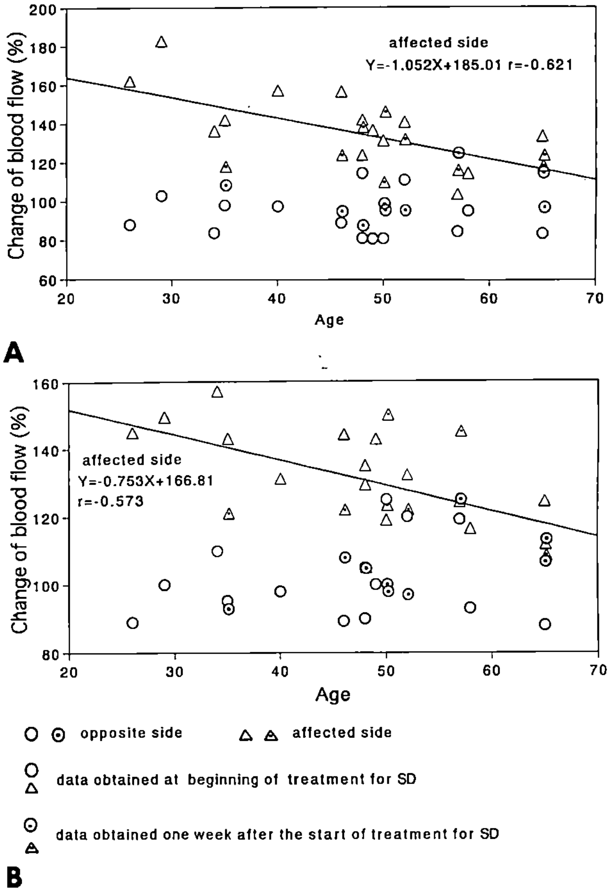

Fig 5. Relationship, after stellate ganglion block, between age of patient and change of blood flow in A) common carotid artery and B) vertebral artery.

Sudden deafness is believed to be caused by various morbid states in the inner ear such as a circulation disorder, virus infection, etc. Since basic and clinical studies on cerebral ischemic diseases have focused on hemorheological factors, the same approach has attracted attention in otological research on SD. Our clinical studies indicate that blood viscosities are significantly greater in patients with SD than in healthy controls and that there is a positive correlation between average hearing level and blood viscosity in SD patients before treatment.[4] In the present study, we found a negative correlation between F and blood viscosity in patients with SD in both the CCA and the VA on the affected side. A similar correlation was present on the opposite side and in controls. This means that blood viscosity constitutes one of the determinant factors of blood flow. In Fig 4, there were a few patients whose hearing loss improved yet whose blood flow decreased on the affected side. In most of these patients, their pretreatment F showed relatively high levels and the decreased levels during treatment remained above the average of normal controls. Therefore, decrease of the blood flow in CCA and VA could not be an etiologic factor of hearing disorder.

The effect of SGB on blood flow has been described in several reports. Kamimura[5] measured blood velocity, blood flow, and diameter of the CCA in adult males, using an ultrasonic blood flow measurement device, before and after SGB, and found that the average increases in each parameter were 54%, 75%, and 7%, respectively. Ono et al[6] reported an increased blood flow of 19% in the CCA and 38% in the ophthalmic artery in men after SGB. The experiment of Mineta et al[7] with dogs showed that the distal portion of the CCA receives sympathetic innervation from the superior and middle cervical ganglia, the middle portion from the superior and middle cervical ganglia and the stellate ganglion, and the proximal portion from the middle cervical ganglion and the stellate ganglion. These portions are controlled to some extent bilaterally, but mostly ipsilaterally. Although the carotid artery has been studied well, there is a paucity of information on the VA system in relation to SD.

Recently, Ren et al[8] reported that SGB blocks postganglionic fibers from the stellate ganglion to both cochleas and preganglionic fibers that make contact with neurons at synapses in the superior cervical ganglion that supply the ipsilateral cochlea. It is generally held that blocking of the preganglionic fibers results in decentralization that makes it hard to respond to systemic stress. On the other hand, if the postganglionic fibers are cut, peripheral receptors could become excessively responsive to noradrenaline generated by stress in other parts of the body and secreted into the blood. It may be rational to assume that SGB is important as treatment to augment local blood flow. In the present study, F in the CCA and VA on the affected side increased after SGB. This increase could be influential in increasing secondarily the blood flow in the cochlea. This effect could supplement the above-mentioned effect of decentralization.

The present study made clear that the F in the CCA on the opposite side decreased after SGB. Since cardiac output remains unchanged after SGB,[9,10] the increased amount of blood flow in the CCA and VA of the affected side may reduce the amount of blood flow in the CCA of the opposite side. So, one should watch closely for ischemia in the perfusion area of the opposite CCA. Moreover, the changes of F in the CCA and VA after SGB became less with age. Nakajima[11] gradually raised the water pressure in the lumen of aortas extirpated from men at autopsy and measured the increase of the diameter to determine the extent of hardening of the aortic wall. He concluded that the maximum degree of dilatation decreased with age. It has also been established that the response of blood vessels to β-receptor stimulation decreases with age. However, one study has shown that the contractile reaction of blood vessels to α-receptor agonists is maintained in older men and older rats, but another report states that it decreases with age. Thus, it can be stated that the reaction of the CCA and VA to SGB varies with age, mainly because of physical changes in the arterial wall and of responsiveness to sympathetic nerve receptors in the arterial wall. This concept should be applicable in the treatment of SD.

## SUMMARY

Blood flow was measured in the CCA and the VA by the ultrasonic Doppler method in 14 male patients with SD and 70 normal adults.

In patients with SD, the F on the affected side was lower than that on the opposite side or that of the control group. Although these differences were not statistically significant in the CCA or VA, a difference in F was found between the SD group with a hearing loss of greater than 50 dB and the SD group with a loss of less than 50 dB. A negative correlation was found between blood viscosity and F in both CCAs and both VAs.

After SGB, the mean V, F, and A of the CCA and VA increased on the side of the SGB and decreased on the opposite side. The changes of F after SGB decreased with age, presumably because of changes in the blood vessel walls and a weaker response to sympathetic nerve receptors in the arterial wall.

ACKNOWLEDGMENTS — The authors thank Dr K. Sugita, Dr T. Negishi, and Dr M. Kawaguchi for their assistance in this study.

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## PEDIATRIC OTORHINOLARYNGOLOGY

The Seventh International Congress of Pediatric Otorhinolaryngology will be held in Helsinki, Finland, June 7-10, 1998. For more information, contact Pekka Karma, MD, Chairman, or Hans Ramsay, MD, Secretary, ENT Department, Helsinki University Central Hospital, Haarmaninkatu 4 E, FIN-00290 Helsinki, Finland; telephone 358 9 4713019; fax 358 9 4715010; E-mail ramsay@hansram.pp.fi.

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## THE SEVENTEENTH EUROPEAN INSTRUCTIONAL COURSE ON TINNITUS AND ITS MANAGEMENT

The Seventeenth European Instructional Course on Tinnitus and Its Management will be held in Nottingham, England, on April 13-17, 1997. For information, contact Mrs Eileen Moxon, Centre for Continuing Professional Development, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; telephone +44 (0115) 951 3700; fax +44 (0115) 951 3722; Email Eileen. Moxon@nottingham.ac.uk.

*Ann Otol Rhinol Laryngol* 106:1997

# POSTNATAL CHANGE IN ANGLE BETWEEN THE TYMPANIC ANNULUS AND SURROUNDING STRUCTURES

## COMPUTER-AIDED THREE-DIMENSIONAL RECONSTRUCTION STUDY

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Postnatal developmental relationships in human ears were studied by a computer-aided three-dimensional reconstruction and measurement method. We measured the angle, in reference to the horizontal plane, between the tympanic annulus, the oval window, and the internal auditory canal (IAC) in 20 normal temporal bones obtained from individuals between 1 day old and 76 years old. The horizontal plane was defined as the plane sloped 30° infero-anteriorly from the plane of the horizontal canal in each specimen. The plane of the tympanic annulus changed from a nearly horizontal orientation (34.2° from the horizontal plane) in neonates to a more vertical orientation (63.3° from the horizontal plane) in adults. The tympanic annulus and oval window planes remained at the same angle to each other (11.9° ± 5.1°) throughout postnatal development, as did the plane of the tympanic annulus and the IAC (68.6° ± 5.3°). These findings have implications for ear surgeons, especially those operating on young children.

KEY WORDS — computer-aided three-dimensional reconstruction, horizontal plane, internal auditory canal, measurement, oval window, postnatal development, tympanic annulus.

## INTRODUCTION

The tympanic membrane is one of the most important landmarks for middle and inner ear surgery, and after tympanoplasty it is very important that the tympanic membrane be replaced in its normal position. Thus, information about the angular relationship between the tympanic membrane and other clinically significant surrounding structures such as the oval window and the internal auditory canal (IAC) would be helpful. The present study was undertaken to obtain this information.

## MATERIALS AND METHODS

For this study, we used 20 temporal bones from the collection of the Elizabeth McCullough Knowles Otopathology Laboratory in the Department of Otolaryngology, University of Pittsburgh. Specimens were from 11 males and 9 females 1 day to 76 years old at death. None of the individuals had a known history of congenital anomaly or otologic disease. All specimens were processed for histologic study according to the technique described by Sando et al,[1] and reference marks were made in each celloidin block to permit accurate alignment for computer-aided three-dimensional (3-D) reconstruction of the tissues. The embedded specimens were sectioned serially either horizontally or vertically, and every 5th to 10th 30-μm-thick section was stained with hematoxylin and eosin.

The equipment used for computer-aided 3-D reconstruction and measurement consisted of a personal computer (NEC 98-RL, model 5) with a frame buffer (Sapience HyperFrame +) for full-color images, a color monitor (16.7 million colors, 640 × 400 pixels), a digitizer (Hitachi HDG-1515) with resolution set at 0.1 mm, and a printer. The software we used was developed by Takagi and Sando[2] and then revised by Fujita and Sando[3] to provide a surface model image.

With the series of histology sections and the computer system described above, the tympanic annulus, oval window, IAC, and semicircular canals were reconstructed in three dimensions for all cases. For some cases, the ossicles, facial nerve, chorda tympani nerve, and round window were also reconstructed. The longitudinal axis of the IAC axis was defined as the line between the center of the orifice and that of the fundus. Then, four planes were defined by choosing three representative points for each. The plane of the horizontal semicircular canal was defined by three well-separated points along the center of the canal. The horizontal plane of the specimen was then

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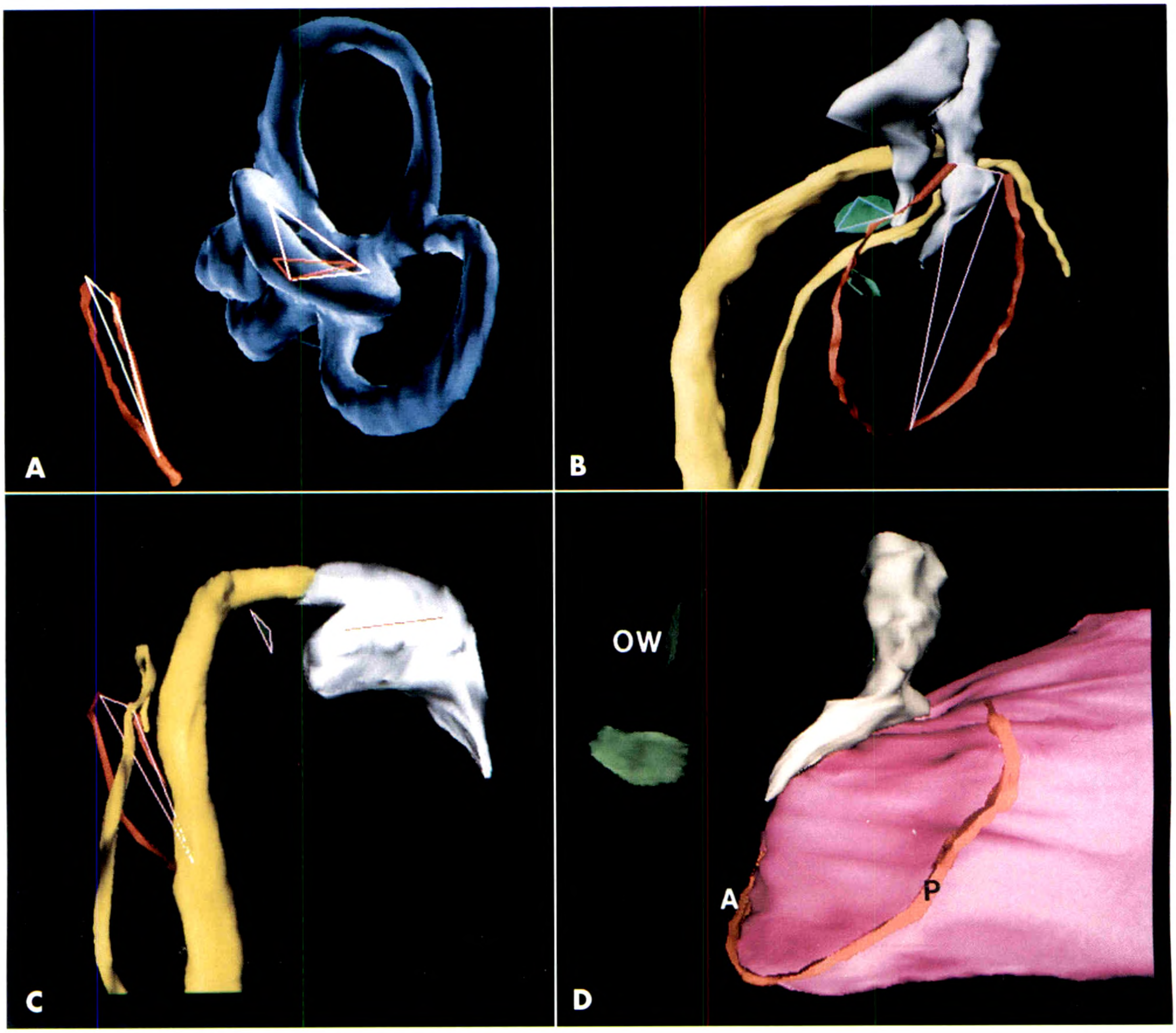

Fig 1. A) Spatial relation of tympanic annulus (red) and semicircular canals (blue) of normal left ear of 10-year-old boy viewed from posterolaterally. Red triangle — horizontal plane, blue triangle — tympanic annulus plane, pink triangle — horizontal canal plane. B) Lateral view of tympanic annulus (red), oval window (green), round window (green), ossicles (gray), facial nerve (thick yellow), and chorda tympani nerve (thin yellow) of normal right ear of 40-year-old woman. Blue triangle — oval window plane, pink triangle — tympanic annulus plane. C) Spatial relation of tympanic annulus (red), internal auditory canal (IAC; gray), oval window (green), facial nerve (thick yellow), and chorda tympani nerve (thin yellow) of normal left ear of 10-year-old boy viewed from posteriorly. Red line — IAC axis, small pink triangle — oval window plane, large pink triangle —tympanic annulus plane. D) Posterior view of tympanic annulus (red), external auditory canal (pink), malleus (gray), oval window (OW; green), and round window (green) of normal right ear of 76-year-old man. Viewed from this direction, which is almost parallel to plane of oval window, oval window appears as thin slit. Posterior portion (P) of tympanic annulus can be seen to be more lateral than anterior portion (A) of tympanic annulus; thus, plane of tympanic annulus is not quite parallel to plane of oval window.

defined as the plane tilted 30° infero-anteriorly from the horizontal canal plane.[4] The tympanic annulus plane was defined by three points along the annulus: the two points immediately adjacent to the greater and lesser tympanic spines and the point at the bottom of the annulus that gave the best fit to define a plane representing the tympanic annulus. The oval window plane was defined similarly by three points chosen to best represent the plane of this structure. The following measurements were then made: 1) the angles between the horizontal plane and the tympanic annulus plane (Fig 1A), oval window plane, and axis of the IAC, 2) the angle between the tympanic annulus plane and the oval window plane (Fig 1B), and 3) the angle between the tympanic annulus plane and the axis of the IAC (Fig 1C). Age-related trends were assessed by correlation coefficient between measurements and log10 of age.

## RESULTS

Measurements of the 20 temporal bones showed that the angle between the plane of the tympanic

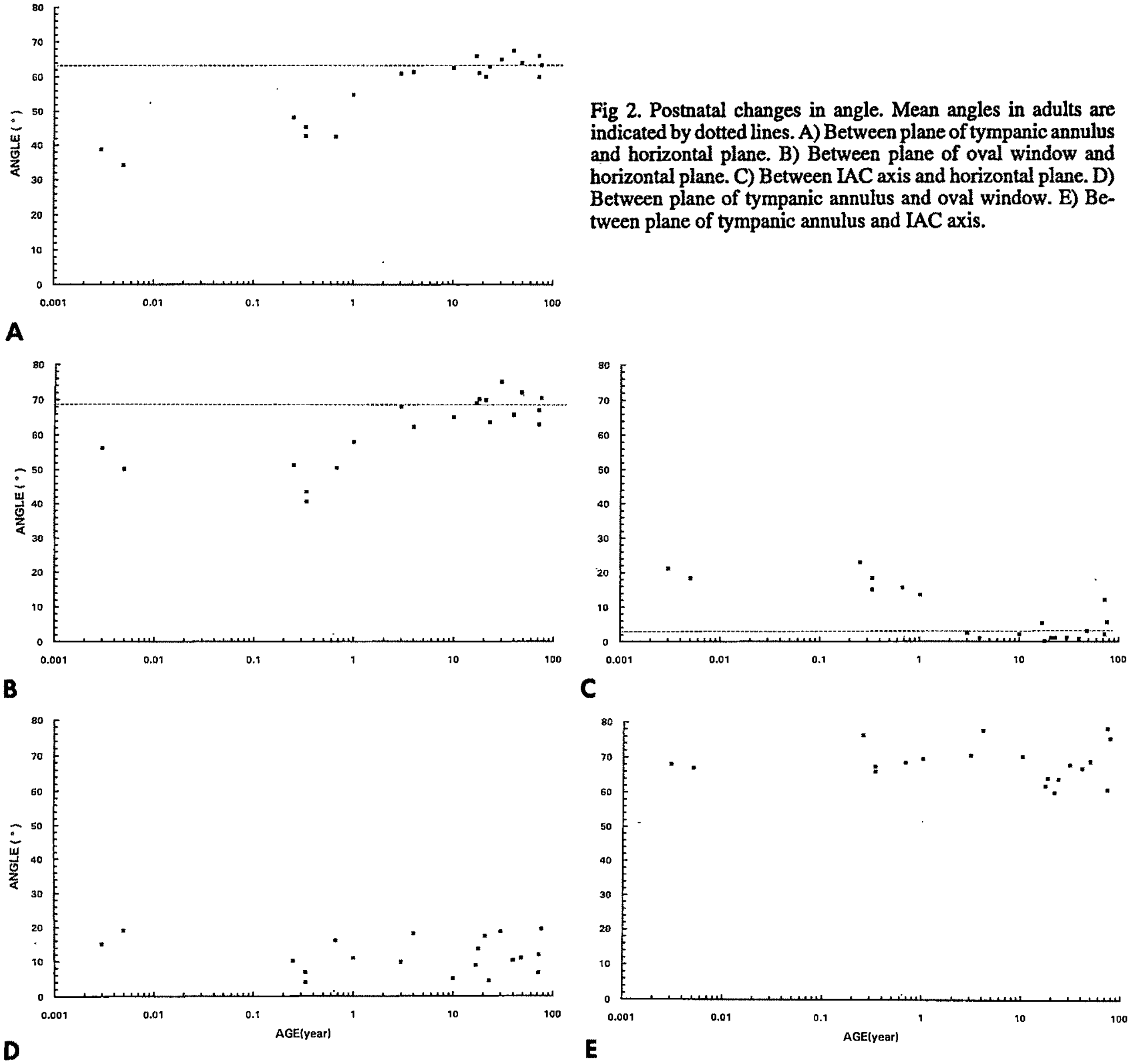

Fig 2. Postnatal changes in angle. Mean angles in adults are indicated by dotted lines. A) Between plane of tympanic annulus and horizontal plane. B) Between plane of oval window and horizontal plane. C) Between IAC axis and horizontal plane. D) Between plane of tympanic annulus and oval window. E) Between plane of tympanic annulus and IAC axis.

annulus and the horizontal plane increased from 34.2° to 61.0° in our specimens (n = 8) during the first 3 postnatal years ($r = .8512$, $p < .01$), and that the angle at 3 years of age is essentially the same as that maintained during adulthood (63.3° ± 2.6°, n = 9; Fig 2A). The angle between the plane of the oval window and the horizontal plane also increased during the first 3 years of life, from 41° to 68° ($r = .7491$, $p < .03$; Fig 2B). However, the angle between the IAC axis and the horizontal plane decreased, from 23° to 2.5° ($r = .9361$, $p < .001$; Fig 2C) during the same period. In our adult specimens (n = 9) the angle between the oval window and horizontal plane averaged 68.5° ± 4.0° and the angle between the IAC axis and horizontal plane averaged 2.9° ± 3.8°. The angle between the plane of the tympanic annulus and oval window (mean ± SD 11.9° ± 5.1°, range 4.5° to 19.5°; Fig 2D) and the angle between the tympanic annulus plane and the axis of the IAC (mean ± SD 68.6° ± 5.3°, range 61° to 79°; Fig 2E) did not vary with age. In all 20 cases the posterior portion of the tympanic annulus was tilted away from (more lateral to) the oval window (Fig 1D).

## DISCUSSION

*Angle Between Tympanic Annulus Plane and Horizontal Plane.* Textbooks state that the angle between the tympanic membrane and horizontal plane increases with age.[5,6] However, the literature does not describe the methods used to determine the horizontal plane and the plane of the tympanic membrane to define this relationship. Because the inner ear reaches its final configuration during fetal life,[7] we used the plane of the horizontal semicircular canal as the basis for measurements made for this study. The plane of the tympanic membrane can be represented by the plane of the tympanic annulus. Using the plane of the horizontal semicircular canal to define the horizontal plane in each specimen, we found that at birth the plane of the tympanic annulus lies relatively close (34.2°) to the horizontal. After birth the plane of the tympanic annulus becomes more vertical, reaching the adult configuration of 63.3° to the horizontal plane during the first 3 years of life. Most of this shift probably results from lateral movement of the tympanic ring (especially its inferior portion) that accompanies growth of the tympanic cavity (unpublished observations) and mastoid.[8] The significant change in the angle of the tympanic membrane (annulus) during the first 3 years of life suggests that the age of the child is an important consideration in planning middle ear surgery on infants and young children.

*Angle Between Tympanic Annulus Plane and Oval Window Plane.* This study revealed that the angle between the plane of the tympanic annulus, which becomes more vertical as age increases, and the oval window plane does not change postnatally (range 4.5° to 19.5°; Fig 2D). The stability of the relationship between these two structures is due to both planes becoming more vertical with increasing age (Fig 2A,B), which in turn may result from postnatal enlargement of the middle ear cavity (tympanic space) and development of the stapes.[9] The oval window lies at the junction between the middle and inner ear; the plane of the oval window is influenced by the configuration of both the middle and inner ear structures. Thus, developmental changes in the oval window plane may be a sensitive indicator of growth of the middle ear.

Several findings of this study can be of help during otologic surgery. If the tympanic annulus is difficult to identify during tympanoplasty (for example, because of previous surgery or severe disease such as cholesteatoma), the surgeon may locate the tympanic membrane by finding the oval window, which our study shows is almost in the same plane in patients of any age. Conversely, during stapes operations, the plane of the tympanic annulus can serve as a reference for the plane of the oval window. Surgeons may also wish to bear in mind that the posterior edge of the tympanic annulus is always more lateral to the oval window than its anterior edge (Fig 1D).

*Angle Between Tympanic Annulus Plane and Internal Auditory Canal.* The results of our study show that the angle between the plane of the tympanic annulus, which becomes more vertical as age increases, and the IAC axis does not change postnatally. This finding is consistent with a previous report that with increasing age, the tympanic annulus plane becomes more vertical and the IAC axis more horizontal.[10] In addition, the IAC (especially its medial part) lengthens significantly until about 10 years of age, but the diameter of the fundus and the lengths of the lateral part of the IAC, transverse crest, and Bill's bar are unchanged.[11] These findings all suggest that postnatal changes of the IAC axis may be due mainly to growth of the bone around the otic capsule These discoveries suggest that for patients of any age, the plane of the tympanic annulus and the axis of the IAC are excellent references for each other. This information can be useful for radiologic evaluation and during otologic surgery.

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ACKNOWLEDGMENTS — The authors thank Eugene N. Myers, MD, Professor and Chairman of the Department of Otolaryngology, University of Pittsburgh School of Medicine, for his encouragement to study this subject, and Marcia C. Williams for her assistance in this study.

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*Ann Otol Rhinol Laryngol* 106:1997

# SECRETORY OTITIS MEDIA IN ADULTS: II. THE ROLE OF MASTOID PNEUMATIZATION AS A PROGNOSTIC FACTOR

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The sequelae of secretory otitis media (SOM) were monitored in 72 adult patients with SOM who were followed up for an average of 33 months. It was found that SOM became chronic and retraction of the tympanic membrane appeared as a function of the pneumatization of the mastoid. Ears with poor pneumatization (less than 6 cm$^2$) developed chronic SOM in 52.2% of cases, as compared with 20% in cases with well-pneumatized ears (6 cm$^2$ and above). Atelectasis developed in 37.3% of poorly pneumatized ears, and in only 5.7% of well-pneumatized ears. These sequelae may therefore be linked pathogenetically to the extent of pneumatization, as both the SOM and the sequelae appeared many years after formation and maturation of the pneumatic system. This study supports other studies that view the mastoid pneumatic system as an organ, as a middle ear pressure buffer. Well-pneumatized ears rarely develop a negative pressure and are seldom associated with chronic sequelae. Ears with poorly pneumatized mastoids lack the physiological function of such a pressure buffer. Ears with a tendency to develop a negative gas balance, whether as a result of deficient ventilation or excessive diffusion, will therefore develop a negative pressure more readily when their pneumatic system is underdeveloped, and consequently will be more prone to develop chronic sequelae.

KEY WORDS — acute otitis media, atelectasis, cholesteatoma, chronic otitis media, mastoid, mastoid pneumatization, middle ear pressure, retraction pockets, secretory otitis media.

## INTRODUCTION

In our previous study[1] we found that adult ears with poor mastoid pneumatization constitute a high-risk group for the development of secretory otitis media (SOM). This conclusion is consistent with previously published findings regarding the relationship between the extent of pneumatization and the risk of development of chronic otitis media[2] and SOM in children.[3] In the present study we examined the correlation between the size of adult mastoid pneumatization and the probability that an adult ear with SOM will develop into chronic SOM or retraction of the tympanic membrane (ie, atelectasis).

## PATIENTS AND METHODS

*Patients.* The study population consisted of 72 adult patients, ages 14 to 80 years (mean 49 years). As mentioned in our earlier article,[1] among the 72 patients, 30 (41.67%) had bilateral SOM and 42 had unilateral SOM. Only those patients in whom SOM was a recent episode during their adult life (postpubertal) and only the 102 ears with SOM were enrolled in this study. The analysis of the distribution of the pneumatization among patients with unilateral and bilateral SOM is presented in the earlier article.[1] Patients with barotrauma or nasopharyngeal carcinoma were excluded, as were those who had undergone ear surgery or had a history of atelectasis. The medical histories, unilaterality and bilaterality, and state of the nasopharynx in these patients have been previously described.[1] Patients were followed up for an average of 33 months (range 17 to 48 months).

*Treatment and Course of Disease.* Patients were usually treated with antibiotics for up to 4 weeks; thereafter, if the SOM did not resolve, they were offered insertion of a ventilating tube (VT). Thirty-one patients were so disturbed by the hearing loss that they opted for immediate insertion of a VT.

Patients in whom the SOM resolved either spontaneously without insertion of a VT or after extrusion of the first VT were considered to have had mild (or acute) SOM. Patients in whom SOM persisted after extrusion of the first VT, and who therefore required more than one VT, were defined as having chronic SOM. The average duration of the persistence of a VT before extrusion was 7 months, and the mean duration of the VT tube was found to be uncorrelated with whether the present VT insertion was a first or a recurrent insertion.

*Measurement of Mastoid Pneumatization.* As in our earlier study,[1] ears were divided into two groups according to the state of their mastoid pneumatization. Group A consisted of 67 ears; 7 were sclerotic, 47 were diploic, and 13 had a pneumatized area of less than or equal to 6 cm$^2$ on planimetric measure-

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DEVELOPMENT OF CHRONIC SOM AND ATELECTASIS IN EARS WITH POORLY PNEUMATIZED (GROUP A) AND WELL-PNEUMATIZED (GROUP B) MASTOIDS

| | *Group A* *(n = 67)* | | *Group B* *(n = 35)* | | *Total* *(n = 102)* | |
|---|---|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* | *No.* | *%* |
| Acute or regular SOM | 67 | 65.7 | 35 | 34.3 | 102 | 100.0 |
| Chronic SOM | 35 | 52.2 | 7 | 20.0 | 42 | 41.2 |
| Atelectasis | 25 | 37.3 | 2 | 5.7 | 27 | 26.5 |

SOM — secretory otitis media.

ment.[2-5] Group B consisted of 35 ears with well-pneumatized mastoids of 7 to 30 cm$^2$. The resolution of whether an ear with SOM developed chronic SOM or atelectasis was performed prior to the evaluation of the size of pneumatization and independently of that evaluation.

## RESULTS

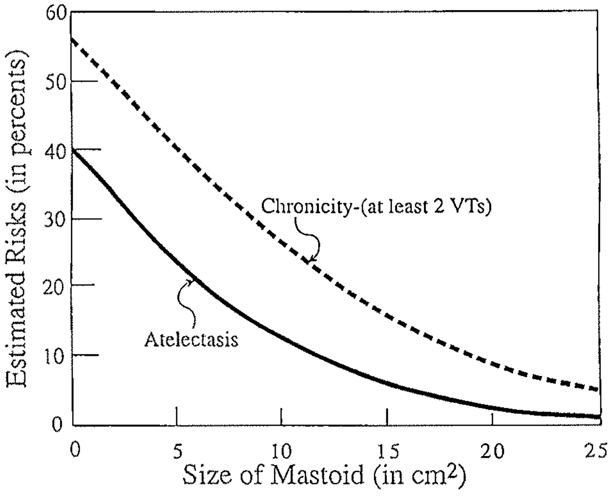
*Comparisons Between Pneumatization in Patients With Acute and Chronic SOM and in Patients With and Without Atelectasis.* As shown in the Table, of the 67 ears in group A (poorly pneumatized mastoids), 35 (52.2%) developed chronic SOM, while 25 (37.3%) became atelectatic (grades I through III). In contrast, of the 35 ears in group B (pneumatized area 7 to 30 cm$^2$), only 7 (20%) developed chronic SOM and 2 (5.7%) became atelectatic (grades I through III). For both chronic SOM and atelectasis, the differences between the groups were highly significant ($p < .001$).

In patients with acute SOM, the mean (±SD) pneumatized area was 7.0 ± 7.23 cm$^2$ as compared with 3.14 ± 3.87 cm$^2$ in patients with chronic SOM. In addition, the mean pneumatized area in the patients who did not develop atelectasis was 6.47 ± 6.88 cm$^2$, as compared with 2.48 ± 3.04 cm$^2$ in those who developed atelectasis. In both cases, the differences between the means were highly significant ($p < .001$).

The mean pneumatization level for all patients in this study (5.41 ± 6.31 cm$^2$) was significantly lower than that of normal patients (10.04 ± 5.0 cm$^2$).[6]

Thus, the tendency of adult patients with acute SOM to develop chronic SOM or atelectasis, like the tendency of normal adults to develop acute SOM,[1] is strongly correlated with poor pneumatization.

*Estimation of Risk by Logistic Regression.* Logistic regression models[7] were used to analyze and estimate the risk of developing chronic SOM or atelectasis as a function of the extent of pneumatization in adults with SOM. In both cases, a good fit was obtained, and as expected, the coefficients of the area of pneumatization were negative; ie, the risk of developing chronic SOM or atelectasis decreases with increasing size of the air cell system. The estimated log odds functions were $-0.37 - 0.16X$ for developing atelectasis and $+0.25 - 0.13X$ for developing chronic SOM, where $X$ is the level of pneumatization. The estimated risk values are presented in the Figure. We emphasize that the estimation of the parameters in the logistic model was based on *all* the 102 observations (from groups A and B) combined, thus yielding low standard errors for the estimates.



Estimated risks (in percent) of development of chronic secretory otitis media (SOM) or atelectasis after SOM episode as function of degree of pneumatization. Note: smaller degree of pneumatization correlates with higher chance of chronic SOM and atelectasis development. VTs — ventilation tubes.

The estimated risks for groups A and B are obviously identical with those estimated from the empirical data. Thus, the estimated risk of developing atelectasis is 6.5 times greater for patients in group A than in group B (37.3% versus 5.7%), and the risk of developing chronic SOM is 2.6 times greater in group A than in group B (52.2% versus 20.0%). These risk ratios are for adult patients with acute SOM. In the normal population, the ratios are much higher. In our study of normal adults,[1] the risk of developing SOM was about 8 times greater in those with poor pneumatization (group A) than in those with adequate pneumatization (group B). On the basis of those findings and the results of the present study, it can be seen that the risk of developing chronic SOM (as a sequela of acute SOM) is 21 times greater in group A patients than in adults with good pneumatization, and that the risk of developing atelectasis is more than 50 times greater in patients from group A than in adults with good pneumatization.

## DISCUSSION

Our previous study[1] demonstrated that a poorly pneumatized mastoid (less than 6 cm$^2$) constitutes a significant risk factor for the development of acute

SOM. The results of the present study indicate that following an inflammatory episode such as acute SOM, the same cutoff point, ie, 6 $cm^2$ of mastoid pneumatization, is seen to be related to the prognosis with regard to the future aeration of the middle ears, namely, the development of chronic SOM or of atelectasis of the tympanic membrane.

It is interesting that in spite of numerous communications that tie the size of the mature mastoid pneumatization to the future well-being of the tympanic membrane, this relationship is usually ignored. The relationship between the size of the mastoid air space and the prognosis of the middle ear condition was first discussed by Flisberg et al[8] and thereafter by Holmquist and Bergstrom.[9] Holmquist[10] also reported that the outcome of ear surgery was related to the size of the mastoid pneumatization. This was also observed in cases of postoperative atelectasis, which fared worse when mastoidectomy was performed concomitant with tympanoplasty[11] than in cases where the mastoid was left intact. In the same context, the poor results of tympanoplasty in children reported by Bonding[12] and Bonding and Satage[13] may also be attributed to the yet-underdeveloped mastoids. Poor pneumatization was shown to be related to an unfavorable prognosis of acute otitis media by Quarnberg[14] and of SOM by Bloch and Feuerstein,[15] Sadé and Hadas,[16] and Lindman and Shea.[17] Finally, tympanic membrane retraction was even observed to appear after stapes surgery in ears with poorly pneumatized mastoids.[18]

There are five factors that together determine the middle ear pressure: 1) ventilation through the eustachian tube, 2) gas diffusion into the circulation, 3) thickness of the middle ear mucosa, 4) elasticity of the tympanic membrane, and 5) size of mastoid pneumatization. It is the role of mastoid pneumatization that this study tries to clarify. Poorly pneumatized mastoids appear to represent an anatomic condition predisposing toward the development of SOM in normal adults[1] and toward chronic SOM and atelectasis in adults suffering from an episode of SOM (sometimes a first episode). This sequence is probably related to the physiological role of the pneumatized mastoid as an organ that regulates or buffers ear pressure irregularities. The physiological role of the pneumatized mastoid can be seen to be a function of its volume, which, according to Boyle's law, may share and damp middle ear pressure aberration. Control of middle ear pressure aberration by the mastoid air cell system was beautifully demonstrated experimentally by Richards et al.[19]

Negative pressure is a general feature of the various forms of otitis media,[20-22] and its proper regulation (or buffering) apparently cannot occur in the absence of adequate pneumatization of the mastoid. The actual origin of such negative pressure is not completely clear, but we see from this study that an inflammatory condition such as SOM can trigger or set in motion a process that will result in a negative gas balance in the middle ear, possibly through ventilation deficiency or increased diffusion.[23] When a negative gas balance is coupled with poor pneumatization, the middle ear is subjected to a negative pressure that over the years may lead to retraction of the tympanic membrane and finally even to cholesteatoma. Poor pneumatization, which is almost always associated with such chronic conditions,[4,8,24] may be viewed as the background on which the vicious circle of negative pressure develops. Retractions of the tympanic membrane and other chronic conditions of the middle ear (eg, central perforations, atelectasis, and cholesteatomas) are generally accepted as sequelae of SOM.[25-27] The modus operandi of atelectasis and its related conditions as sequelae of SOM[25] is usually attributed, without any explanation, to the traditional mythical concept of "eustachian tube deficiency." The present study, as well as several other studies, suggests that sequelae usually attributed to SOM may actually be related to poor pneumatization.

The question of whether mastoid pneumatization is mainly determined genetically or is secondary to any environmental factors (inflammation) has not been addressed in this study; both may play a role in determining the final size of the mastoid.

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## INNER EAR MEDICINE AND SURGERY

The Sixth Symposium and Workshops on Inner Ear Medicine and Surgery, combined with the Prosper Meniere Society Annual Winter Meeting, will be held in Aspen/Snowmass, Colorado, March 22-29, 1997. For further information, contact Prosper Meniere Society, c/o Jane Wells, Meeting Coordinator, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

*Ann Otol Rhinol Laryngol* 106:1997

# LARYNGEAL ADJUSTMENT IN WHISPERING

## MAGNETIC RESONANCE IMAGING STUDY

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Previous studies confirmed that during whispering the glottis is kept open to prevent vocal fold vibration and the supraglottal structures are constricted. However, there has been no study exploring the exact contour of the laryngeal lumen in the frontal dimension during the production of whispering. In order to further elucidate the nature of the laryngeal adjustments regarding the contour of the laryngeal lumen in whispering, and the role of supraglottal constriction in particular, we conducted a physiological study using magnetic resonance imaging. According to the results, the supraglottal structures were not only constricted but also shifted downward, attaching to the vocal fold to prevent vocal fold vibration completely during whispering. The results suggested the underlying mechanism of suppression of vocal fold vibration during the production of whispering.

KEY WORDS — laryngeal shape, larynx, magnetic resonance imaging, physiology, whispering.

## INTRODUCTION

Whispering is an aphonic laryngeal action.[1] This means that there is no vocal fold vibration during whispering. Tsunoda et al[2] elucidated the nature of the physiological condition for whispering, with special attention to the laryngeal adjustments. Their results indicated that for the production of whispering there existed a necessary relationship between the glottal and supralaryngeal adjustments in terms of the coordination between the posterior cricoarytenoid muscle, contributing to glottal abduction, and the thyropharyngeus muscle, contributing to supraglottal constriction. Other, previous studies[3-5] also confirmed that during whispering, the glottis was kept open to prevent vocal fold vibration, and that the supraglottal structures were constricted to produce a turbulent noise for whispering.

However, there has been no study exploring the exact contour of the laryngeal lumen in the frontal dimension during the production of whispering. In order to further elucidate the nature of the laryngeal adjustments regarding the contour of the laryngeal lumen in whispering, and the role of supraglottal constriction in particular, we conducted a physiological study using magnetic resonance imaging (MRI).

## MATERIAL AND METHODS

The subjects were three native Japanese men. All of them were healthy volunteers without any disease in the larynx or respiratory system. The subjects were required to produce five sustained Japanese vowels in isolation. They uttered each vowel in ordinary phonation, whispered phonation (the kind of whisper used when whispering into someone's ear), and stage whispering (that which one would use if the listener were quite a distance away from the speaker). Each phonation was performed separately for more than 4 seconds, in order to obtain a clear image. During each utterance, an MRI scan was performed with a Hitachi MRI system (1.5 T). For the purpose of observing the contour of the laryngeal lumen in frontal section, the MRI section was made approximately perpendicular to the vocal fold at the middle of the membranous portion of the vocal fold.

## RESULTS

Figure 1 illustrates an MRI sample obtained from subject 1 producing a sustained Japanese vowel /o/ in ordinary phonation, in whispering, and in stage whispering.

As shown in Fig 1, both vocal folds were adducted

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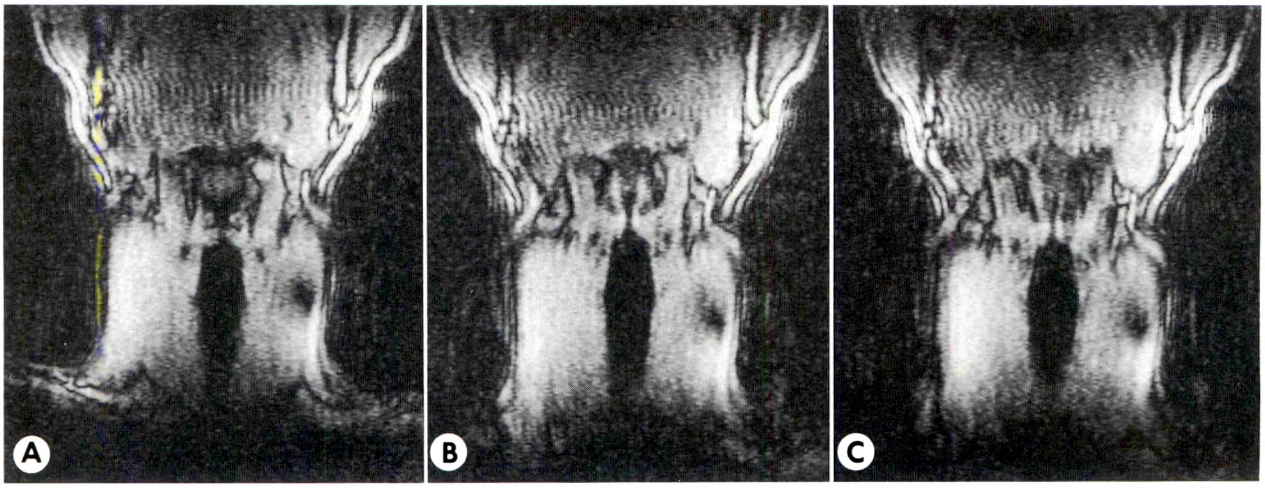

Fig 1. (Subject 1) Sustained vowel /o/ in A) ordinary phonation, B) whispering, and C) stage whispering.

and the glottis was closed in the midline in ordinary phonation. The false vocal fold was clearly identifiable, which was separated from the vocal fold by the air-filled space of the laryngeal ventricle. On the other hand, in both modes of whispering, the laryngeal ventricle appeared to be narrowed, and the supraglottal structures — the false vocal folds in particular — shifted downward, impinging on the vocal folds. The glottal closure appeared to be incomplete in both modes of whispering, and there was a clear glottal chink at the level of the vocal folds.

A similar difference in laryngeal configuration was observed in the other subjects. Figure 2 shows an example from subject 2 producing a sustained Japanese vowel /a/ in ordinary phonation, in whispering, and in stage whispering.

Figure 3 shows an MRI sample obtained from subject 3 producing a sustained Japanese vowel /o/ in ordinary phonation, whispering, and stage whispering. In this subject, it appears that there was a tendency toward supraglottal constriction in the anterior-posterior dimension during the production of whispering, which is characterized by the contour of the epiglottis. Incidentally, there was an apparent glottal chink even during ordinary phonation in subject 3.

## DISCUSSION

It has been reported that the laryngeal gestures during whispering observed laryngoscopically from above were characterized by supraglottal constriction associated with incomplete closure of the glottis.[2-5] Our previous electromyographic study revealed that the thyropharyngeus muscle contributed to the supraglottal constriction in whispering, being activated twice as forcefully in stage whispering as in soft whispering, and with tighter constriction than in soft whispering.[2]

The result of the present study suggested that the supraglottal structures were not only constricted but also shifted downward, impinging on the vocal fold to completely prevent vocal fold vibration during whispering, while the same structures were separated

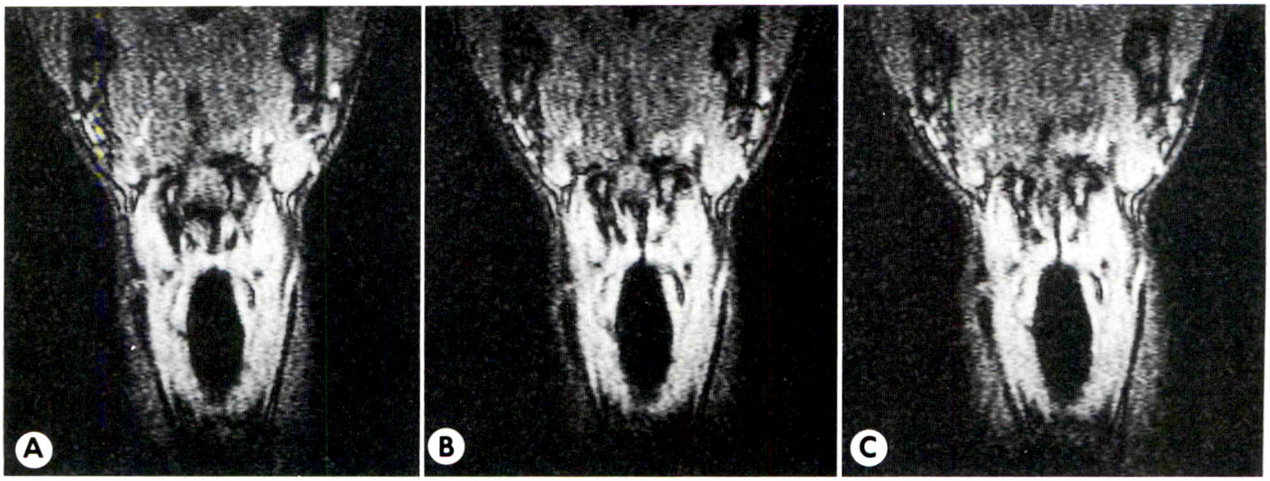

Fig 2. (Subject 2) Sustained vowel /a/ in A) ordinary phonation, B) whispering, and C) stage whispering.

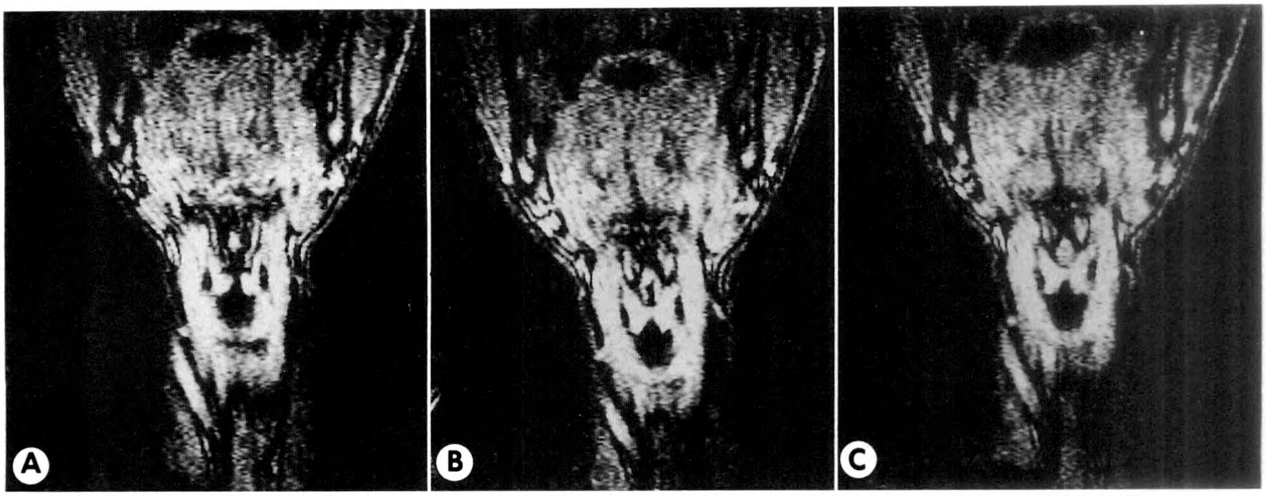

Fig 3. (Subject 3) Sustained vowel /o/ in A) ordinary phonation, B) whispering, and C) stage whispering.

from the vocal folds during ordinary phonation.

Although whispering is characterized by aphonic laryngeal action, it is also associated with a turbulent noise produced at the glottis. An aerodynamic study[4] on whispering indicated that the airflow rate is twice as high in soft whispering and three times as high in stage whispering as in ordinary phonation. It should be reasonable to assume that the constriction of the supraglottal structures plays the role of a brake system of vocal fold vibration during whispering against the stronger airflow. In this sense, the brake system has to work more strongly in stage whispering, because of its higher airflow, to provide a tight constriction.

## CONCLUSION

In conclusion, the present study using MRI analysis suggested the underlying mechanism of suppression of vocal fold vibration during the production of whispering.

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ACKNOWLEDGMENTS — We thank the medical staff of Hitachi General Hospital, Department of Radiology, particularly Dr Koutaro Nakajima and Kazuya Sukegawa, for advice on magnetic resonance imaging.

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*Ann Otol Rhinol Laryngol* 106:1997

# AGE-RELATED CHANGES OF ELASTIC FIBERS IN THE SUPERFICIAL LAYER OF THE LAMINA PROPRIA OF VOCAL FOLDS

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An investigation was carried out to determine the morphologic characteristics of elastic fibers in the superficial layer of the lamina propria of aged vocal folds (EFAVFs). Excised human adult vocal folds served as the material for this study. Scanning and transmission electron microscopic observations were made. The results can be summarized as follows. First, the EFAVFs were composed of amorphous substances and microfibrils. The amorphous substances increased in amount and the microfibrils became less numerous. Second, the EFAVFs ran in various directions, were branched, and formed a complicated network. The surface of the fibers was rough, and the fibers appeared to vary in size. Some EFAVFs united to form a sheet with a rough surface. Third, the EFAVFs could not be easily digested by elastase compared with those of younger adults. We conclude that the morphologic and metabolic changes of elastic fibers in the most important vibrating portion (superficial layer of the lamina propria) of the aged vocal folds contribute partially to aging of the voice.

KEY WORDS — aging of vocal folds, aging of voice, elastic fibers, vocal folds.

## INTRODUCTION

The human voice changes with age.[1] Some histologic changes in the vocal fold tissue have been related to the voice's aging.[2,3] The exact mechanisms of this aging, however, have not yet been clearly determined. The most important part of the vibrating structure of the vocal fold is the cover, which consists of the epithelium and the superficial layer of the lamina propria.[4] Geriatric changes in the fine structure of the superficial layer of the lamina propria are supposed to be one of the important causes of the voice's change with aging.

The superficial layer of the lamina propria is composed of fibrous components (elastic and collagenous fibers) and ground substance.[5] The elastic fibers give viscoelasticity to the tissue. The elastic fibers are composed of microfibrils and amorphous substances, and amorphous substances consist of elastin protein.[6,7] Elastase is the enzyme that decomposes the elastin,[6,8] and it plays an important role in the metabolism of elastic fibers.

In this study we focused on the age-related changes of elastic fibers in the superficial layer of the lamina propria of the aged vocal fold. First, we investigated age-related morphologic changes of elastic fibers by electron microscopy. Second, we conducted an elastase digestion study to investigate age-related metabolic changes in elastin and elastic fibers.

## MATERIALS AND METHODS

We used 10 normal human adult larynges obtained from autopsy cases: 5 from aged people with ages ranging from 74 to 87 (3 men and 2 women), and 5 from younger adults with ages ranging from 34 to 39 (2 men and 3 women). One vocal fold of each larynx was investigated by scanning electron microscopy (SEM), and the other by transmission electron microscopy (TEM).

For SEM, each specimen was fixed in 2.5% glutaraldehyde at 4°C for 2 hours. After rinsing with cacodylate buffer solution, the collagen fibers were chemically digested by the modified sodium hydroxide maceration method.[9] All specimens were immersed in 6N sodium hydroxide for 10 minutes at 65°C, washed in physiological saline solution, and postfixed in 2% osmium tetroxide with Millonig's phosphate buffer solution[10] at 4°C for 2 hours. This was followed by dehydration in a graded series of concentrations of ethanol, immersion in *tert*-butyl alcohol, and drying by the *tert*-butyl alcohol freezing method. The specimens were then sputter-coated with gold and examined under a Hitachi S-800 scanning electron microscope.

For TEM, each specimen was fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution, and postfixed in 2% osmium tetroxide with Millonig's phosphate buffer solution[10] at 4°C for 2 hours. They were then dehydrated in a graded series of concentrations of ethanol and embedded in epoxy resin. Semithin sections were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope.

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Presented at the 3rd International Symposium on Phonosurgery, Kyoto, Japan, June 26-28, 1994.

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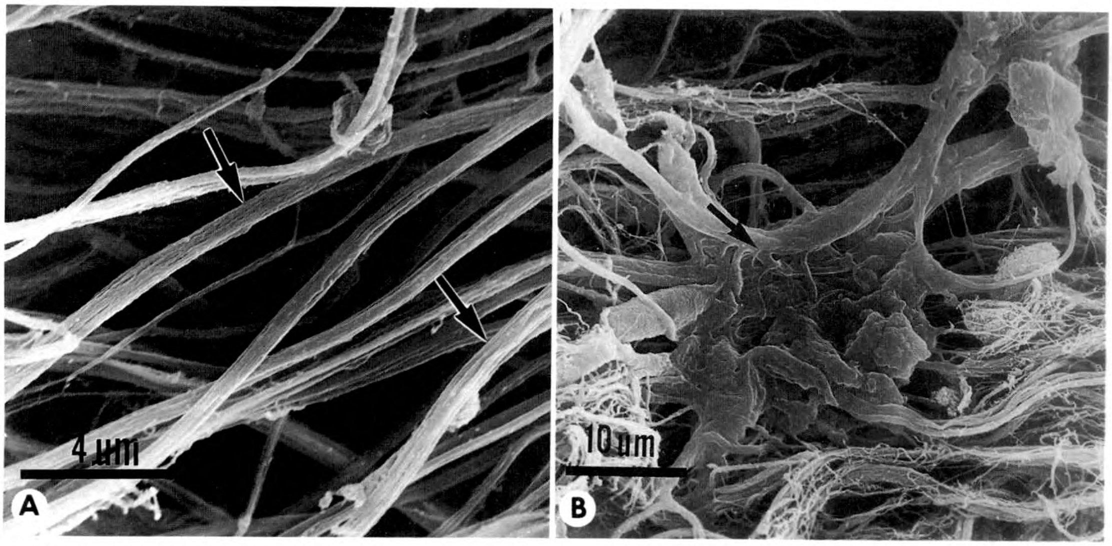

Fig 1. Scanning electron micrographs of elastic fibers in superficial layer of lamina propria of vocal fold. A) Younger adult. Arrows — elastic fibers. B) Aged adult. Arrow — some elastic fibers united to form sheet with rough surface.

Thin sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate, and tannic acid to make elastin apparent. The sections were then observed under a JEM-2000EX transmission electron microscope.

For the elastase digestion study, the epoxy resin–embedded sections were used.[8] One milligram of porcine pancreatic elastase (Wako Pharmaceutical Co, Osaka, Japan) was diluted in 10 mL of phosphate-buffered saline. Thin sections were prepared with an ultramicrotome and incubated in the diluted elastase solution for 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours at 37°C. All the thin sections were subsequently stained with tannic acid for elastin contrast. Observation was made with a JEM-2000 EX transmission electron microscope.

## RESULTS

*Scanning Electron Microscopic Study*. Figure 1 shows SEM findings for elastic fibers in the superficial layer of the lamina propria. Elastic fibers alone remained following treatment by the modified sodium hydroxide maceration method.

The elastic fibers of the younger adults (Fig 1A) were aligned in essentially the same direction, parallel to the direction of the vocal fold edge. They were slender and branched and anastomosed to form a loose network. Spaces among the fibers were relatively large. The surface of the fibers was smooth and its shape was uniform. The fibers were cylindrical or elliptical in shape and the diameter was about 0.5 µm.

In the aged vocal folds (Fig 1B), the elastic fibers ran in various directions, were branched, and formed a complicated network. The spaces between the elastic fibers were smaller than those in the younger adults. The surface of the fibers was rough, and the fibers appeared to vary in size. Some elastic fibers united to form a sheet with a rough surface (Fig 1B, arrow).

*Transmission Electron Microscopic Study*. Figure 2 shows TEM findings for elastic fibers in the superficial layer of the lamina propria. The elastic fibers comprised microfibrils and amorphous substances (Fig 2). Many microfibrils were present along the periphery of elastic fibers in the younger adult vocal folds (Fig 2A). In the aged vocal folds (Fig 2B,C) the fibers were larger and rougher than those in the younger adult vocal folds. The amorphous substances increased in amount and the microfibrils became less numerous. Some of the fibers in the aged vocal folds had become fragmented (Fig 2D).

*Elastase Digestion Study*. The results for this study are given in the Table. The elastin in the elastic fibers in the superficial layer of the lamina propria of the aged vocal folds was difficult to digest compared with that of the younger adults.

Elastic fiber elastin from the younger adults started to undergo digestion by elastase in 30 minutes, and more than 75% was digested in 2 hours. That from aged specimens did not undergo digestion uniformly. Digestion started in 2 hours and was more than 75% complete at the end of 4 hours.

Figure 3 shows elastic fibers undergoing digestion by elastase. The amorphous substances showed a marked affinity for tannic acid and stained black. Staining was reticular from the periphery of the fibers at the start of digestion. Reticularly stained portions

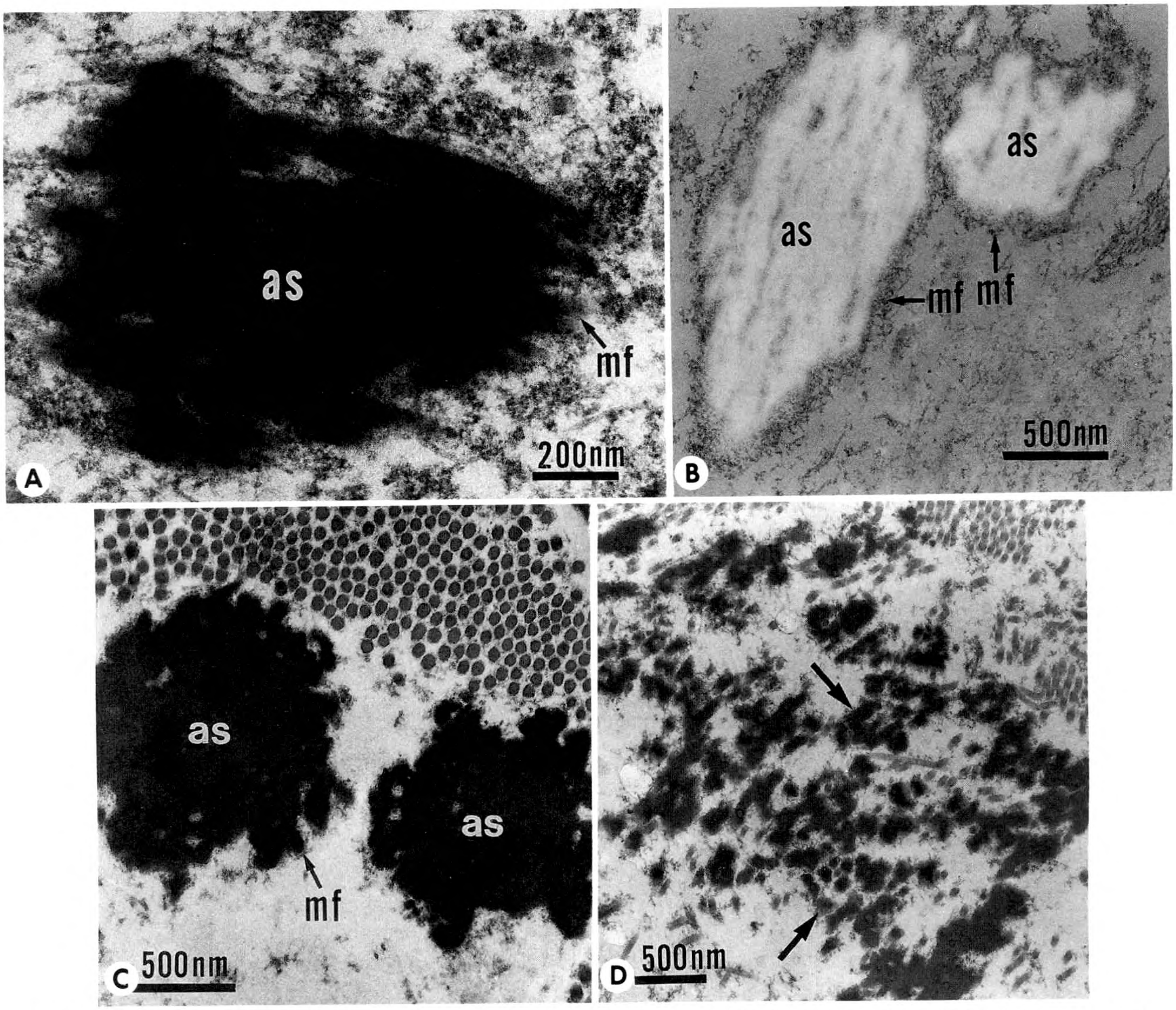

Fig 2. Transmission electron micrographs of elastic fibers in superficial layer of lamina propria of vocal fold. A) Younger adult (tannic acid stain). Many microfibrils (mf) are present along periphery of fibers. as — amorphous substances. B) Aged adult (uranyl acetate and lead citrate stain). Amorphous substances (as) have increased in amount, and microfibrils (mf) have become less numerous. C) Aged adult (tannic acid stain). Amorphous substances (as) have increased in amount, and microfibrils (mf) have become less numerous. D) Aged adult (tannic acid stain). Arrows — fragmented fibers.

increased and digestion finally proceeded to completion.

## DISCUSSION

Histologic changes in the vocal fold mucosa are one of the important causes of voice aging.[2,3] There have been some previous reports on age-related changes in the elastic fibers of the vocal fold mucosa. Hirano et al[2] noted the elastic fibers in the intermediate layer of the lamina propria to become less dense and atrophy in men, and such changes were less marked in women. Sato et al[3] carried out a light microscopic investigation of elastic fibers in the superficial layer of aged vocal folds and observed them to be degenerated, atrophic, and irregularly fragmented.

The most important vibrating portion of the vocal folds during phonation is the cover, consisting of epithelium and the superficial layer of the lamina propria.[4,5] Histologic changes in this layer are one of the important causes of the change in voice quality with aging. The edematous change in the superficial layer of the aged lamina propria is well known.[2] Our previous light microscopic investigation on elastic fibers in the superficial layer of the aged lamina propria revealed morphologic changes.[3]

From the physiological and biomechanical point of view, elastic fibers have very important functions. Elastic fibers can be stretched to their breaking point at about 150% of their original length with a force of only 20 to 30 kg/cm$^2$,[11] thus demonstrating the sufficient resilience they give to tissue. Elastic fibers in the superficial layer of the lamina propria contribute

RESULTS OF ELASTASE DIGESTION STUDY

| | *30 min* | *1 h* | *2 h* | *3 h* | *4 h* |
|---|---|---|---|---|---|
| Elastic fibers in aged adults | – | – | + | ++ | +++ |
| Elastic fibers in younger adults | + | ++ | +++ | | |

– — no digestion, + — less than 25% digestion, ++ — 25% to 75% digestion, +++ — more than 75% digestion.

to the stiffness and viscoelasticity of the vocal fold mucosa.

*Morphologic Changes of Elastic Fibers.* Our electron microscopic investigation revealed that the elastic fibers in the superficial layer of the aged lamina propria were changed morphologically compared with those of younger adults. The SEM study showed that elastic fibers in the superficial layer of the aged lamina propria ran in various directions, were branched, and formed a complicated network; the surface of the fibers was rough; and the fibers varied in size. These findings suggested that elastic fibers could not give sufficient resilience to the tissue. According to the TEM study, the elastic fibers in the superficial layer of the aged vocal folds consisted of greater amounts of amorphous substances, and microfibrils were fewer in number. The microfibrils constitute the predominant structure of developing elastic fibers.[7] The elastic fibers in the superficial layer of the aged lamina propria have thus ceased to develop and have changed morphologically.

*Metabolic Disorder of Elastic Fibers.* Elastic fibers are composed of elastin protein. Elastase decomposes the elastin[6,8] and is related to the metabolism of elastin and elastic fibers. Our investigation revealed that the elastin in elastic fibers in the superficial layer of the aged lamina propria could not be digested easily by pancreatic elastase, compared with that of younger adults. This finding indicated that the metabolism of the elastic fibers had changed. The activity of serum elastase decreases with aging.[12] The turnover and metabolism of elastic fibers in the superficial layer of the aged lamina propria are suggested to occur more slowly.

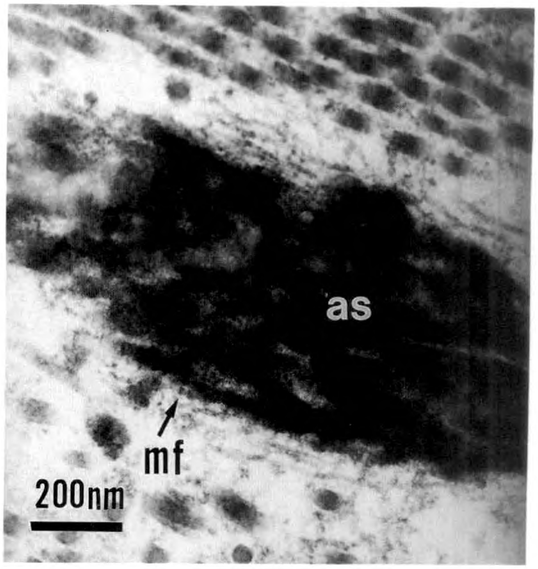

Fig 3. Transmission electron micrograph of elastic fibers undergoing digestion by elastase (tannic acid stain). Staining of tannic acid (black) is reticular from periphery of fibers. mf — microfibrils, as — amorphous substances.

*Decreased Synthesis of Elastic Fibers.* Our previous investigation revealed that the fibroblasts in the adult macula flava control the synthesis of fibrous components in the vocal fold mucosa.[13] The decrease in number and the inactivation and degeneration of fibroblasts in aged macula flava indicate decreased synthesis of fibrous components in the vocal fold mucosa.[14] From this point of view, newly synthesized elastic fibers in the superficial layer of the lamina propria decrease in aged vocal fold mucosa.

Elastic fibers in the superficial layer of the lamina propria of aged vocal folds change morphologically, and metabolism of these fibers is disturbed. So, their turnover and repair become slow. Furthermore, newly synthesized elastic fibers decrease. These elastic fibers appear to lose their functions, giving insufficient resilience to the tissue. The morphologic and metabolic changes of elastic fibers in the tissue most important for vibration (superficial layer of the lamina propria) of the aged vocal fold contribute partially to aging of the voice.

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ACKNOWLEDGMENTS — We thank Dr Yuuji Ito and Dr Yasuhiro Nakamura, St Mary's Hospital, Kurume, for their thoughtful contribution of the cadaver larynges.

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*Ann Otol Rhinol Laryngol* 106:1997

# MECHANICS OF TYPE IV TYMPANOPLASTY: EXPERIMENTAL FINDINGS AND SURGICAL IMPLICATIONS

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In a type IV tympanoplasty, the stapes footplate is directly exposed to incoming sound while the round window is "shielded," usually with a fascia graft. Postoperative hearing results are quite variable, with air-bone gaps ranging from 10 to 60 dB. A cadaveric human temporal bone preparation was developed to investigate the middle ear mechanics of this operation to identify causes of variable results and to test predictions of a recently described theoretic model of type IV tympanoplasty. The ear canal, tympanic membrane, malleus, and incus were removed so as to expose the stapes and round window to the sound stimulus. A "cavum minor" chamber (air space adjacent to the round window) was constructed around the round window niche. The round window could be isolated from sound by placing an acoustic shield over this chamber. The mechanical properties of the shield, cavum minor, annular ligament, and round window membrane were varied experimentally. Stapes velocity as determined by an optical motion sensor was used as a measure of hearing level. The largest stapes velocity occurred with a mobile stapes and round window, a stiff shield, and a well-aerated cavum minor. Partial fixation of the stapes or round window caused a decrease in stapes velocity. Acoustic shields of conchal cartilage or Silastic silicone rubber sheeting (approximately 1 mm thick) provided near-optimal shielding. A temporalis fascia shield resulted in a stapes velocity 10 to 20 dB less than that seen with a cartilage or Silastic silicone rubber shield at low frequencies. A cavum minor air space as small as 16 μL was sufficient for unrestricted stapes motion, provided the air was in contact with the round window membrane. These results qualitatively matched predictions of our model, but there were some quantitative differences. The clinical implications of our results are that in order to optimize postoperative hearing, the surgeon should 1) preserve normal stapes mobility, preferably by covering the footplate with a very thin split-thickness skin graft, not a fascia graft; 2) reinforce a fascia shield with cartilage or Silastic silicone rubber; 3) create conditions that promote aeration of the round window niche; and 4) preserve the mobility of the round window membrane.

KEY WORDS — middle ear mechanics, middle ear model, temporal bone measurements, type IV tympanoplasty.

*This paper is dedicated to the memory of Harold F. Schuknecht, MD, a superb and gifted otologist, otopathologist, scholar, and teacher. His teachings and scientific contributions profoundly influenced the practice of otology in the latter half of this century, and it was a privilege and honor for us to have come under his tutelage.*

## INTRODUCTION

Type IV tympanoplasty is a well-established technique used to reconstruct ears with canal wall–down mastoid cavities when the tympanic membrane, malleus, incus, and stapes superstructure are missing or diseased.[1,2] A type IV tympanoplasty involves placing a tissue graft, usually temporalis fascia, to "shield" the round window membrane from sound (Fig 1). The air space that is enclosed between the acoustic shield and the round window is called the cavum minor. The type IV procedure is a simple yet robust alternative to reconstructive techniques using a total ossicular replacement prosthesis (TORP) that avoids the problems of TORP extrusion, rejection, and displacement. However, type IV procedures are not widely performed, perhaps because clinical hearing results are not uniformly satisfactory. Because of loss of the middle ear sound pressure gain produced by the tympanic membrane and ossicles, the expected (theoretic) hearing result after a type IV tympanoplasty is an air-bone gap of 20 to 25 dB.[1,3,4] However, all published series of hearing results after type IV tympanoplasty show a wide range of air-bone gaps (10 to 60 dB), with an air-bone gap of 30 dB or less occurring in only about 50% of cases.[2,4,5] These results are no worse than the results of TORP procedures.[6-8] We feel that the simplicity of the type IV procedure might enable us to determine and control some of the sources of variability in results, thereby increasing the number of cases with acceptable hearing results.

We have developed a cadaveric human temporal bone preparation that allows experimental investigation of the middle ear mechanics of a type IV tympanoplasty. In this preparation, stapes velocity is used as a representative measure of hearing level.[3-5] Our preparation allows us to measure the stapes velocity while manipulating the acoustic properties of four significant structures of the reconstructed middle ear: 1) the stapes footplate and annular liga-

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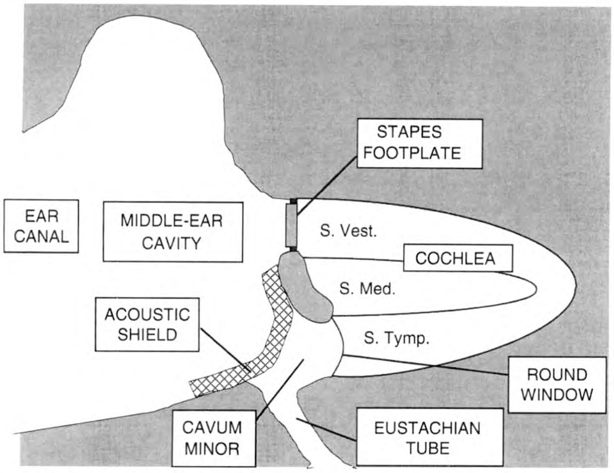

Fig 1. Schematic diagram of type IV tympanoplasty. Tympanic membrane, malleus, incus, and stapes superstructure are missing, and acoustic shield isolates round window from sound in ear canal. Air space between shield, round window, and tympanic orifice of eustachian tube is termed cavum minor.

ment, 2) the round window membrane, 3) the acoustic shield over the round window, and 4) the cavum minor. In this paper, we describe our temporal bone preparation and measurements, and we use our results to specify the functional significance of differences in type IV reconstruction techniques and materials.

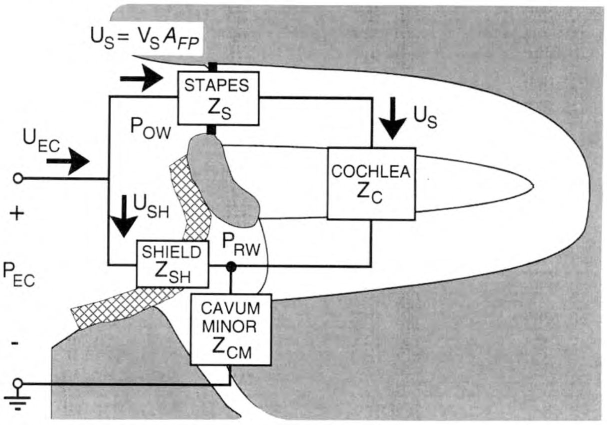

Fig 2. Four-block circuit model of type IV tympanoplasty superimposed over schematic of middle ear anatomy. Four blocks are impedances of 1) stapes and annular ligament $\mathbf{Z_S}$; 2) cochlea and round window $\mathbf{Z_C}$; 3) acoustic shield over cavum minor $\mathbf{Z_{SH}}$; and 4) cavum minor itself $\mathbf{Z_{CM}}$. Sound pressure $\mathbf{P_{EC}}$ and volume velocity $\mathbf{U_{EC}}$ in ear canal produce sound pressure at oval window $\mathbf{P_{OW}}$ and volume velocities of stapes $\mathbf{U_S}$ and shield $\mathbf{U_{SH}}$. Sound pressure at round window $\mathbf{P_{RW}}$ depends on other variables shown. Stapes motion is proportional to difference between $\mathbf{P_{OW}}$ and $\mathbf{P_{RW}}$. Hearing is assumed to be proportional to $\mathbf{U_S}$. $\mathbf{U_S} = \mathbf{V_S}A_{FP}$, where $\mathbf{V_S}$ is stapes velocity and $A_{FP}$ is footplate area.

This experimental preparation also allows us to compare our results with the predictions of a mechano-acoustic model of type IV tympanoplasty[4,5,9] (Fig 2). In the model, stapes velocity $\mathbf{V_S}$ depends on the window pressure difference $\mathbf{P_{WD}}$ between the oval window sound pressure $\mathbf{P_{OW}}$ and the round window sound pressure $\mathbf{P_{RW}}$. The $\mathbf{P_{WD}}$ is influenced by the acoustic impedance of four structures: 1) $\mathbf{Z_S}$, the impedance of the stapes footplate and annular ligament; 2) $\mathbf{Z_C}$, the impedance of the cochlea and round window; 3) $\mathbf{Z_{SH}}$, the impedance of the acoustic shield; and 4) $\mathbf{Z_{CM}}$, the impedance of the cavum minor. (Acoustic impedance $\mathbf{Z}$ is the ratio of sound pressure $\mathbf{P}$ to volume velocity $\mathbf{U}$; volume velocity $\mathbf{U}$ is the product of linear velocity $\mathbf{V}$ and the pertinent cross-sectional area $A$). The details of our model analyses have been described in previous publications.[4,5,9] Qualitatively, the model predicts that the largest stapes velocities will occur when $\mathbf{Z_S}$ and $\mathbf{Z_C}$ are near normal, the cavum minor is aerated such that the magnitude of $\mathbf{Z_{CM}}$ is small compared to the magnitudes of $\mathbf{Z_S}$ and $\mathbf{Z_C}$, and the magnitude of the shield impedance $\mathbf{Z_{SH}}$ is large compared to the impedance of the cavum minor $\mathbf{Z_{CM}}$. The model produces quantitative predictions of postoperative air-bone gaps by using measurements of $\mathbf{Z_S}$ and $\mathbf{Z_C}$ and estimates of the volume of the cavum minor and the acoustic properties of different shield materials. Predictions of postoperative hearing results using realistically attainable acoustic shield values and cavum minor volumes closely match the best clinical type IV surgical results over a broad frequency range (125 to 4,000 Hz).[4,5] Based on these analyses, we have made recommendations regarding suitable shield materials and minimum cavum minor volume .[4,9] In this paper, we use our empirical results to test the predictions of our model, as well as to test and revise our specific recommendations for shield stiffness and cavum minor volume. A further discussion of the mechanical basis of the type IV tympanoplasty model and its implications for the study of middle ear mechanics will be the subject of a separate communication (Ravicz et al, in preparation).

## MATERIAL AND METHODS

### TEMPORAL BONE PREPARATION AND MEASUREMENTS

Measurements were made in seven fresh normal human temporal bones removed at autopsy with an oscillating Stryker bone plug saw. Removal times ranged from 7 to 27 hours postmortem, with a mean of 12 hours. Ages ranged from 42 to 78 years, with a mean of 70 years. Normality of each temporal bone was determined by absence of otologic disease on chart review and by visual inspection of the middle ear and mastoid using an operating microscope. Upon removal, bones were stored in normal saline at 4°C to which a drop of 10% Betadine was added as a bactericide and fungicide (approximately 10 μL of 10% Betadine in 300 mL of normal saline). Bones

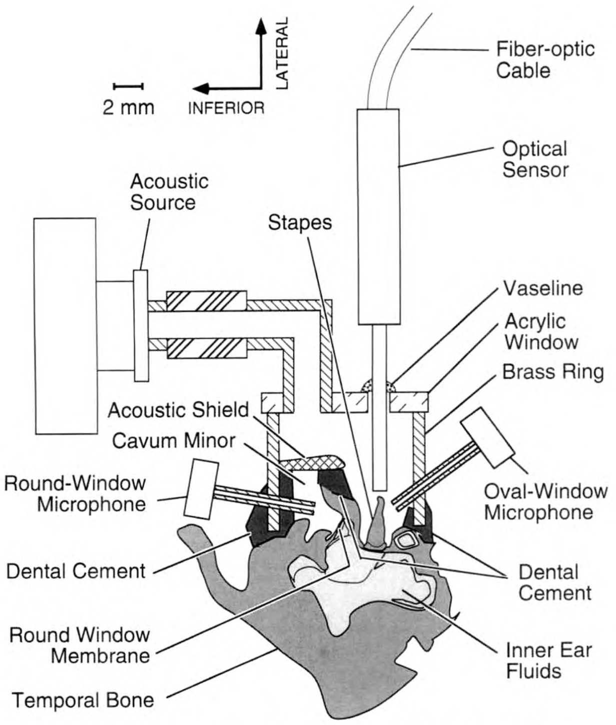

Fig 3. Schematized section of type IV tympanoplasty temporal bone preparation and experimental setup. External auditory canal and mastoid spaces were drilled away, and tympanic membrane, malleus, and incus were removed. Brass ring was cemented to temporal bone to form chamber that encompassed both cochlear windows. Cavum minor was constructed around round window with dental cement. Acoustic source produced sound field within brass ring. Microphones measured sound pressure at oval window **POW** and at round window **PRW**. Different shields could be installed to isolate round window from sound field. Stapes displacement **XS** in response to sound was measured with optical sensor. Openings between acoustic source and brass ring and between optical sensor and acoustic source were sealed with Vaseline.

were then prepared as noted below. The saline-Betadine solution was replaced every 1 to 2 days for the duration of storage. The first measurements in these bones were made 4 to 13 days postmortem. Measurements were repeated in some bones up to 30 days postmortem. Between measurement sessions, bones were stored in the saline-Betadine solution at 4°C.

In each temporal bone, a tympanomeatal flap was first elevated, and the incudostapedial joint was severed with a joint knife while taking extreme care to avoid any manipulation of the stapes. The bony external auditory canal, mastoid air cells, tympanic membrane, malleus, and incus were then removed. The stapedius tendon was severed with alligator-type scissors. The temporal bone was kept moist with periodic applications of normal saline throughout the preparation. Both the oval and round window niches were enclosed in a brass ring (20 mm inside diameter) that was cemented to the medial wall of the middle ear and mastoid (Fig 3). The round window was shielded from sound by constructing a "cavum minor–like" air space around the niche with carboxylate dental cement (Durelon, Germany) and covering its surface with an acoustic shield. The volume of the cavum minor air space was 150 to 300 μL in the various bones. The air volume was measured by filling the cavum minor with saline by means of a calibrated microsyringe. The shape and surface area of the acoustic shield in all the bones were comparable to the in vivo situation. Metal probe tubes (18-gauge, 1.25-mm outside diameter, 0.8-mm inside diameter, 25-mm length) were cemented to the edges of the oval and round window niches. The tip of the oval window probe tube was at the level of the stapes head, while the tip of the round window probe tube was approximately 2 mm away from the round window membrane. The annular ligament and round window membrane were kept moist throughout the measurement sessions by periodic applications of normal saline. The saline was aspirated from the windows just prior to making each measurement.

We made three physical measurements: stapes displacement **XS**, oval window pressure **POW**, and round window pressure **PRW**. (These quantities can be represented as complex numbers that have a magnitude and phase angle. For example, **POW** has a magnitude |**POW**| and a phase angle ∠**POW**.) The **XS**, **POW**, and **PRW** were measured repeatedly in each bone over the frequency range 20 Hz to 8 kHz while the physical properties (impedances) of the annular ligament, acoustic shield, and cavum minor were varied individually (see below). Complete details of the apparatus, instruments used, and technique of measurements have been described previously[10] and are described only briefly here. An acoustic source was coupled to the top of the brass ring, and broadband chirps were delivered to the preparation. Stimulus root-mean-square sound pressures were approximately 80 dB sound pressure level (SPL) for pressure measurements and 130 dB SPL for stapes motion measurements. The **XS** was measured with an optical motion sensor (Ångstrom Resolver 201; Opto-Acoustic Sensors, Raleigh, NC) that was introduced through the acoustic source and oriented perpendicular to the plane of the stapes head. A drop of water-based paint (White-out) was applied to the stapes head to improve its reflectivity. The temporal response of the optical sensor output was averaged, and the magnitude and phase angle of **XS** were calculated at 576 frequency points spaced 24.4 Hz apart by a Fourier transform of the averaged waveform. Stapes linear velocity **VS** was then computed from **XS** by $\mathbf{V_S} = j2\pi f \mathbf{X_S}$, where $j = \sqrt{-1}$ and $f$ = frequency.

Sound pressures at the oval window **POW** and at the round window **PRW** were computed by similar analysis of the output voltages of Knowles EK3027 microphones that were coupled to the metal probe tubes.

### VARIATION OF IMPEDANCES OF ANNULAR LIGAMENT, ACOUSTIC SHIELD, CAVUM MINOR, AND ROUND WINDOW MEMBRANE

The temporal bone preparation allowed us to vary the impedances of the annular ligament, acoustic shield, cavum minor, and round window individually and thereby assess the effect of each modification on **POW**, **PRW**, and **XS**. The following modifications were carried out.

*Annular Ligament*. Allowing the annular ligament to become dry (over a time period of 15 minutes to 2 hours or more) has been shown to cause an increase in its impedance.[10,11] The effect can usually be reversed by remoistening with normal saline. We used this technique as a reversible way of varying stapes impedance. In addition, in one bone, the annular ligament was irreversibly stiffened by application of an alcohol-based solution.

*Acoustic Shield*. A variety of acoustic shields were used, including clay, Silastic (Dow Corning) silicone rubber sheeting (1 mm in thickness), conchal cartilage (approximately 1 mm in thickness), and one or two layers of temporalis fascia (with or without a Teflon Schuknecht crescent). Fascia pieces were remnants from tympanoplasty cases and would otherwise have been discarded. The fascia was stored in normal saline at 4°C and used in the fresh state. Jeltrate impression compound (type II, Dentsply, Milford, Del) was used to form an acoustic seal between the edges of the shield and the top of the cavum minor chamber.

*Cavum Minor*. The volume of air contained within the cavum minor was decreased systematically by partially filling the cavum minor with clay. Finally, the cavum minor was completely filled with degassed normal saline solution, effectively reducing its volume to zero. In some cases, one or more air bubbles of known volume were then introduced into the saline in a controlled fashion.

*Round Window*. The round window impedance was increased by filling the round window niche with Jeltrate, which is somewhat flexible when moist. The Jeltrate was then removed, and the round window was blocked by filling the niche with rigid dental cement.

### VALIDITY OF MEASUREMENTS

Our measurements are based on the following assumptions: 1) The sound pressure measured at the stapes head is similar to the actual sound pressure acting at the level of the stapes footplate. 2) The stapes and cochlea are a linear system at the stimulus levels used for our measurements. 3) The stapes moves as a rigid piston, so that stapes displacement **XS** at the stapes head is similar to that at the stapes footplate. 4) The inner ears used in these experiments remain physiologically similar to living ears, ie, filled with fluid (perilymph and endolymph) with no air bubbles. These assumptions and supporting evidence have been discussed previously.[10]

In the first bone used in this series of experiments (MGH 8A), the stapes superstructure was removed after vaporizing the lower ends of the stapes crurae with a handheld argon laser (brand HGM). Water-based reflective paint was applied to the middle of the footplate for displacement measurements. The paint would remain moist and flexible, generating a large mechanical artifact (see below). Furthermore, the reflective paint would get washed away with every moistening of the annular ligament, making repeated measurements somewhat tedious. In a subsequent bone, displacement and sound pressure measurements made at the stapes head were similar to those made at the footplate for frequencies below 11 kHz. When the stapes was left intact, paint applied to the stapes head remained dry, and mechanical artifact was reduced. Thereafter, a preparation with an intact stapes superstructure was considered to be sufficiently representative of a type IV tympanoplasty, and the stapes was left intact in the remainder of the study.

### STABILITY OF PREPARATION

We have shown previously that measurements in the temporal bone preparation were stable with time.[10] Generally, pressure measurements were extremely stable within sessions or between sessions; variations were approximately 10% (1 dB). Within any one measurement session, the magnitude of stapes displacement |**XS**| generally varied by less than a factor of 2 (6 dB), and its phase angle ∠**XS** varied by less than 0.05 periods (20°); variations between sessions were comparable. In a few bones, there was an inexplicable increase in the stiffness of the annular ligament between sessions. These bones were discarded, and they are not part of the seven bones described in this manuscript.

### MECHANICAL ARTIFACT

Control measurements made on points on the temporal bone near the stapes revealed a mechanical artifact produced by stimulus-linked motion of the entire temporal bone.[10] The magnitude of this artifact was at least 20 dB smaller than the largest stapes motions we measured. However, the artifact placed a lower bound on measurable stapes motion, in that stapes motion smaller than the artifact was undetectable.

## RESULTS

### STAPES MOTION WITH OPTIMAL WINDOW PRESSURE DIFFERENCE

To produce the maximum achievable pressure difference between the oval and round windows, a

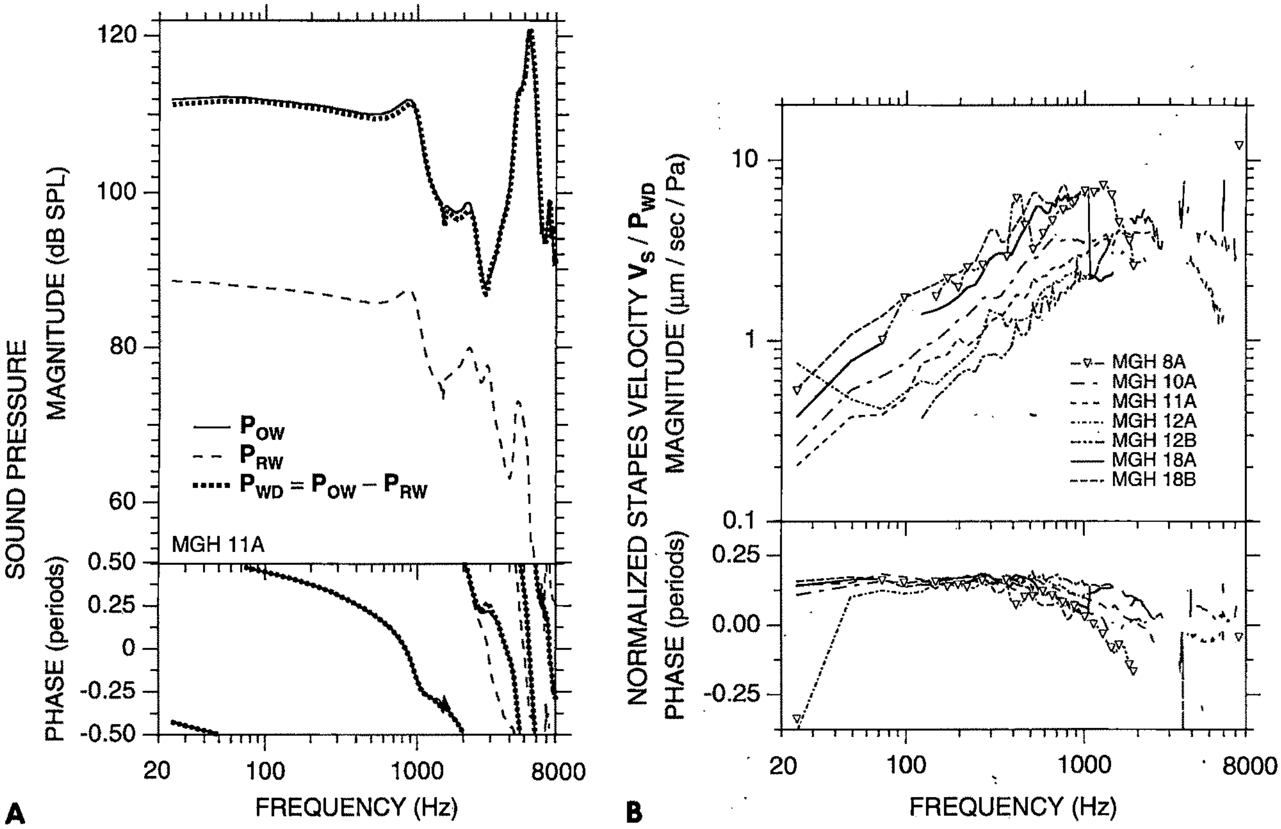

Fig 4. A) Sound pressure at oval window **Pow** (solid line) and round window **Prw** (dashed line) when round window was shielded with 1-mm-thick Silastic silicone rubber. (Sound pressure **P** has both magnitude **|P|** and phase angle **∠P**.) Because **|Prw|** << **|Pow|**, window pressure difference **Pwd** = **Pow** – **Prw** (thick dotted line) is virtually identical to **Pow**. (Phase angles of **Pow** and **Prw** are virtually identical below 2.5 kHz.) B) Mean stapes velocity **Vs**, normalized to window pressure difference **Pwd**, in seven bones. Each curve is mean of *N* measurements made on same day, where *N* = 6,200, 12,000, 11,250, 11,000, 4,250, 3,000, and 5,000 for bones 8A, 10A, 11A, 12A, 12B, 18A, and 18B, respectively. Only those points where **Xs** was at least 15 dB above artifact are shown. **Vs** has several features in common among all bones: 1) Below 1 kHz, **|Vs|** increases as frequency increases, somewhat less than proportionally. 2) Below 500 Hz, **∠Vs** is near +0.125 periods. 3) Above 1 kHz, **|Vs|** is roughly constant with frequency, and **∠VS** is near zero.

stiff cavum minor shield was used to minimize the sound pressure at the round window **PRW**. In some bones, a clay shield reduced **|PRW|** to 40 to 70 dB less than the magnitude of the pressure at the oval window **|POW|**; hence, the window pressure difference **PWD** = **POW** – **PRW** was virtually identical to **POW**. (Recall that the decibel scale is logarithmic [**|P1|** (dB) – **|P2|** (dB) = 20 log (**|P1/P2|**)]; so if **|PRW|** is 40 dB less than **|POW|**, **|PRW|** = 10$^{-2}$ **|POW|**, and **|PWD|** = **|POW** – 10$^{-2}$ **POW|** = 0.99 **|POW|**.) A shield consisting of a 1-mm-thick piece of Silastic silicone rubber sheeting provided 25 to 40 dB of shielding and produced a **PWD** that differed from **POW** by less than 1 dB (10%) and was not significantly different from the **PWD** produced by a clay shield (Fig 4A). The pressures shown in Fig 4A and in subsequent Figures represent those present during the stapes motion measurements.

Figure 4B shows stapes velocity **VS** normalized to the window pressure difference **PWD** in seven bones when the cavum minor was shielded with clay or Silastic silicone rubber. Each **VS** curve shown is the mean of several measurements in that bone. Data points derived from **XS** that was less than a factor of 5 (approximately 15 dB) above artifact levels are not shown. The magnitude of **VS** does not vary much among the seven bones — only about a factor of 5 (approximately 15 dB). The phase angle of **VS** varies by only about 0.05 periods (15°) at low frequencies, but can vary as much as 0.25 periods (90°) above 1 kHz. The measured **VS** is consistent with measurements of stapes-cochlear impedance **ZSC** = **ZS** + **ZC** = **POW/US** in other bones (when **PRW** << **POW**).[10] (**US** = **VS***AFP*, where *AFP*, is footplate area.)

When the acoustic shield was removed, the window pressure difference **PWD** was reduced, and stapes motion was found to decrease as well. With no shield, round window pressure **PRW** was virtually identical to oval window pressure **POW** (both magnitude and phase) at frequencies below 1.5 kHz, as shown in Fig 5A. The magnitude of the resulting window pressure difference **|PWD|** in this frequency range is 20 to 25 dB below **|POW|**. Above 3 kHz, **POW** and **PRW** differ significantly, due to the acoustics of

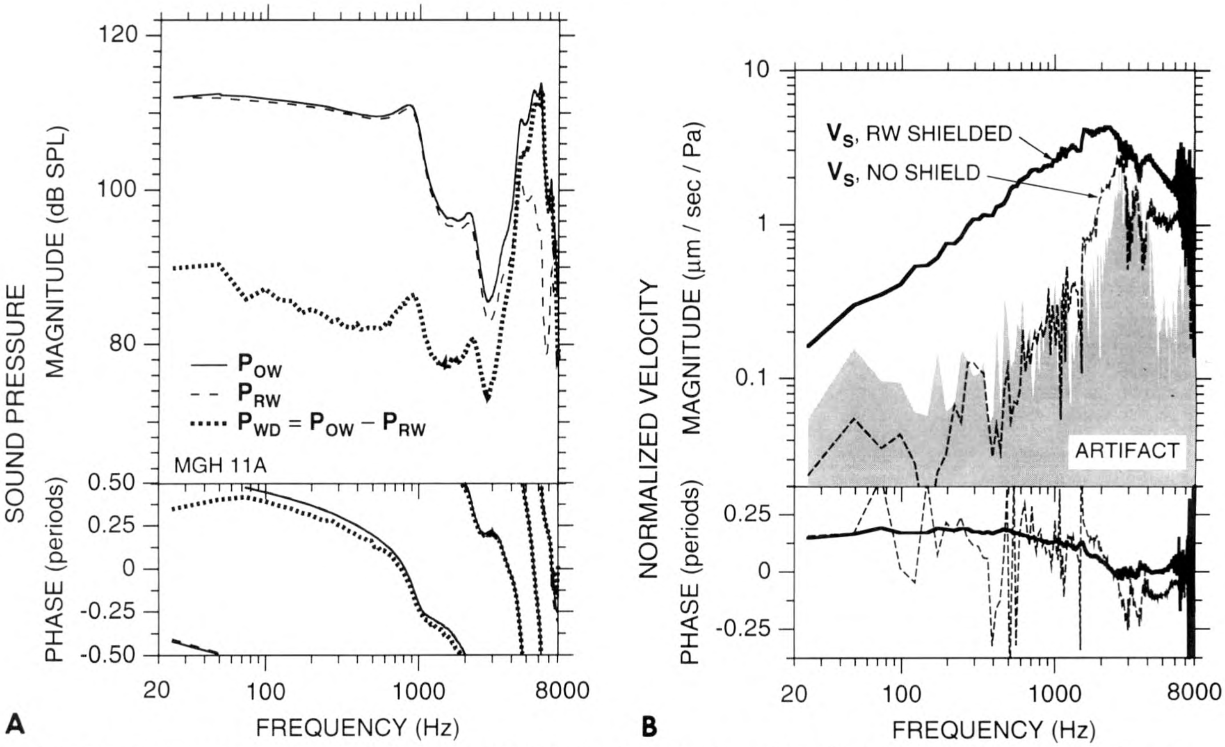

Fig 5. A) Sound pressure at oval window **POW** (solid line) and round window **PRW** (dashed line) when round window was unshielded. Below 1.5 kHz, **PRW** ≈ **POW**, and window pressure *difference* **PWD** = **POW** – **PRW** (thick dotted line) is much less than either **POW** or **PRW**. Above 3 kHz, |**POW**| ≠ |**PRW**|, and **PWD** ≈ **POW**. ∠**POW** and ∠**PRW** are indistinguishable. B) Stapes velocity **VS**, normalized to window pressure difference **PWD**, with round window unshielded (dashed line) versus shielded (solid line). With round window unshielded, |**VS**| is no larger than artifact (shaded area), and phase varies randomly below 1.5 kHz, where |**PWD**| << |**POW**|. At higher frequencies, where **PWD** ≈ **POW**, |**VS**| is larger than artifact.

the preparation: the wavelength of sound approaches the length of the sound path between the two windows, enabling phase and magnitude differences between **POW** and **PRW**. Because of these differences in **POW** and **PRW** at high frequencies, the window pressure difference is no longer small, but instead is similar in magnitude to **POW**. (This dependence of the window pressure difference on frequency was observed by Békésy.[12])

Figure 5B shows stapes velocity **VS** in the shielded and unshielded conditions as well as the mechanical artifact. It is evident that **VS** is reduced substantially when the window pressure difference is reduced. The **VS** is indistinguishable from artifact at frequencies below 2 kHz where |**PWD**| << |**POW**|, but |**VS**| is above the artifact for frequencies above 4 kHz where |**PWD**| ≈ |**POW**|. The reduction in **VS** when **PWD** is reduced is consistent with the predictions of the type IV model.[4,9]

### EFFECT OF VARYING ANNULAR LIGAMENT IMPEDANCE **ZS**

In three bones, the annular ligament was allowed to dry, thereby increasing its impedance. Measurements of stapes velocity **VS** when the annular ligament was dry showed a decrease in |**VS**|, especially at frequencies below 4 kHz (Fig 6). At higher frequencies, stapes motion was relatively unaffected. This decrease in |**VS**| was reversible by remoistening the ligament with saline. The observation that increasing **ZS** affects **VS** most at low frequencies is consistent with model predictions[4,9] and with previous observations[10] that at low frequencies, the impedance of the stapes and cochlea together is due primarily to **ZS**. In one bone, an alcohol-based solution applied to the ligament had an effect on **VS** that was similar in magnitude but irreversible.

### EFFECT OF VARYING SHIELD IMPEDANCE **ZSH**

Various biologic or biocompatible shield materials were tested in two bones to evaluate their effectiveness in shielding the round window from sound: temporalis fascia, auricular conchal cartilage (approximately 1 mm thick), and Silastic silicone rubber sheeting (1 mm thick). The ability of these materials to shield the round window from sound varied; as a result, the window pressure difference **PWD** = **POW** – **PRW** varied for different materials (Fig 7A). Among the materials tested, only Silastic silicone rubber and cartilage had a high enough impedance **ZSH** to provide sufficient shielding such that **PWD** was not significantly different from **POW** at the frequencies measured. One or two layers of temporalis fascia resulted in a **PWD** 10 to 15 dB below **POW** at frequencies below 1 kHz. The Teflon (Schuknecht) crescent added to reinforce the fascia had an insignificant

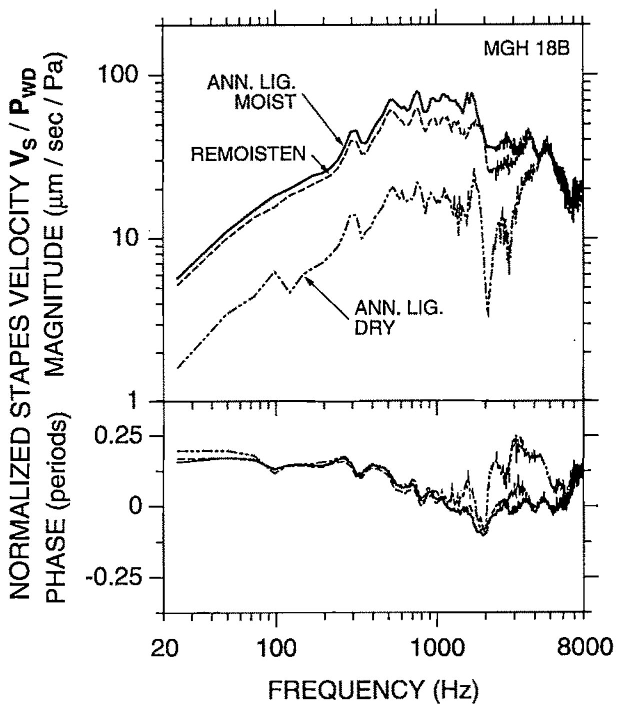

Fig 6. Changes in stapes velocity Vs when annular ligament was allowed to dry out (normalized to window pressure difference PWD). As annular ligament dried, |Vs| decreased (dot-dashed line) from its normal state (solid line). Remoistening annular ligament restored |Vs| to near initial state (dashed line).

effect.

Stapes velocity VS (when normalized by POW) varied in the same fashion as PWD, as shown in Fig 7B. The effects of cartilage and Silastic silicone rubber were similar and resulted in the largest magnitude of VS. With one or two layers of temporalis fascia, |VS| was 10 to 20 dB below that seen with a cartilage or Silastic silicone rubber shield at frequencies below 1 to 2 kHz. In fact, stapes velocity was *proportional* to the window pressure difference (Ravicz et al, in preparation) in that the changes produced in VS by varying the shield are similar to the changes produced in PWD by the same manipulations (eg, a 10-dB change in the magnitude of PWD produces a factor of three difference [10-dB change] in VS; Fig 7). This proportionality would have resulted in identical velocity responses if the velocity data were normalized by PWD as was done in Figs 4B, 5B, and 6. Such proportionality of VS and PWD supports a basic assumption made in the type IV tympanoplasty model (and commonly held in the field of middle ear mechanics), that stapes motion depends on the window pressure *difference* rather than on oval or round window pressure alone.[3,4,9,13,14]

## EFFECT OF VARYING CAVUM MINOR VOLUME AND ZCM

In three bones, stapes displacement was measured while the volume of the cavum minor *VCM* was varied. The impedance of cavum minor ZCM varies inversely with *VCM*, so reductions in *VCM* produce proportionate increases in ZCM. (ZCM = $\rho 0 c^2/[j2\pi f VCM]$, where $\rho 0$ is the density of air = 1.19 kg/m$^3$ and *c* is the speed of sound in air, ie, 345 m/s.[4,15]) The unaltered cavum minor volumes ranged from 170 to 317 µL, somewhat larger than the representative value of *VCM* of 100 µL estimated to exist after a type IV tympanoplasty.[4] The volume was reduced in stages, reaching a minimum value of 16 µL in bone MGH 12A. No significant reduction in stapes displacement was observed after any of these alterations in volume. Technical difficulties with this approach prevented us from being able to produce accurately volumes smaller than 16 µL. However, as demonstrated in the next section, it seems possible that a reduction of cavum minor volume to as little as 2 µL may not cause much decrease in stapes motion at frequencies above 700 Hz.

In five bones, stapes velocity VS was measured with the cavum minor shielded but completely filled with saline. This condition mimics the physiological condition in which the cavum minor is filled with fluid (eg, due to a middle ear effusion). The effect of the saline was to decrease cavum minor volume nearly to zero. Under these circumstances, |VS| was indistinguishable from artifact at frequencies below 4 kHz (Fig 8). Such a reduction is consistent with model predictions.[4,9]

Air bubbles were introduced into the fluid-filled cavum minor of the same saline-filled bones described above. As predicted by the model, an air bubble in the fluid facilitated stapes motion by forming a small cavum minor. A tiny air bubble, on the order of 2 µL, adjacent to and in contact with the round window membrane was sufficient to restore stapes velocity to normal at frequencies above 700 Hz (Fig 9). A bubble of comparable size located in the fluid in the cavum minor but at some distance from the round window membrane could partially restore stapes velocity, but was much less effective. When the bubble was located in areas of the cavum minor away from the round window, larger sizes (approximately 30 µL) were necessary to achieve an effect comparable to that caused by a 2-µL bubble at the round window. In effect, the fluid between the air bubble and the round window membrane *increased* the impedance of the cavum minor and therefore reduced |VS|. This finding, that the *location* of an air bubble within the fluid of the cavum minor was important in determining its efficacy in restoring stapes motion, was not accounted for by the model.[4]

## EFFECT OF RESTRICTING ROUND WINDOW MOTION

The effect of obliteration of the round window by connective tissue and bone was tested in bone MGH 12A. Obliteration by soft fibrous tissue was simulated by filling the round window niche with Jeltrate,

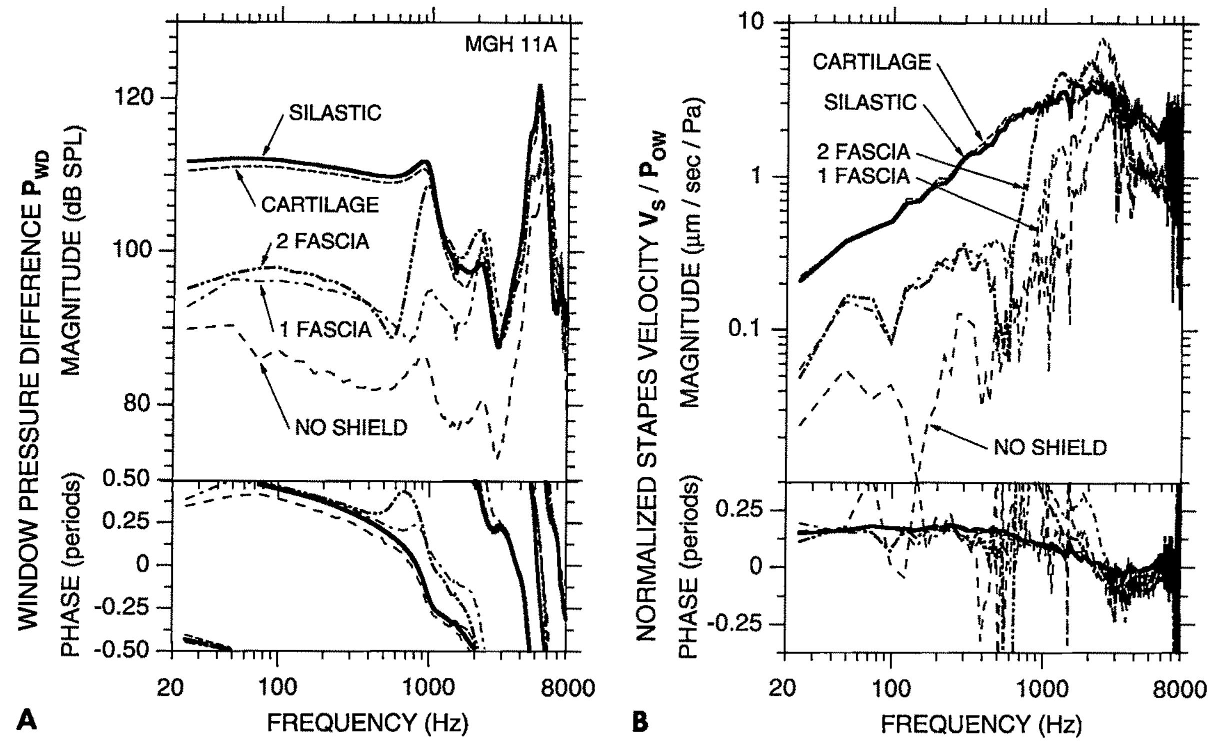

**Fig 7.** A) Window pressure difference PWD = POW – PRW in bone MGH 11A for several shield materials of different impedances. Silastic silicone rubber shield (1-mm thickness) produced window pressure difference (solid line) nearly identical to POW. Cartilage shield produced PWD that was only about 1 dB lower (dashed line). Shield of one or two layers of temporalis fascia produced PWD that was lower by 10 to 15 dB, below 1 kHz (dot-dashed lines). Bottom dashed line is PWD in unshielded case. B) Stapes velocity VS measured in bone MGH 11A while varying acoustic shield material to obtain data in A. All curves are normalized by POW. Silastic silicone rubber or cartilage shield, which produced largest PWD, also produced largest magnitude of VS. |VS| was smaller at low frequencies with fascia shield, and lowest when round window was unshielded. Differences in |VS| between various shields are proportional to differences in |PWD| seen in panel A. Similar results were seen in bone MGH 10A.

which is somewhat flexible when moist. A reduction by a factor of 2 to 3 (6 to 10 dB) in the magnitude of stapes velocity VS was observed across the frequency range (Fig 10). To simulate obliteration by bone, the Jeltrate was removed, and the round window niche was then filled with dental cement, with care being taken to prevent entrapment of air bubbles between the cement and round window membrane. Cement filling reduced stapes motion to a level indistinguishable from artifact. Such a reduction in VS is consistent with the view that the cochlea is incompressible.[13,14]

## DISCUSSION

Several investigators have used a temporal bone preparation to investigate the mechanics of various surgical techniques of middle ear reconstructive surgery.[16-18] The use of temporal bones allows one to make repeated measurements of acoustic and mechanical function after precise modifications that reproduce specific surgical techniques. Most of the modifications can also be reversed, thus allowing close control of the measurements. The temporal bone preparation allows one to test modifications to existing techniques and to test new surgical techniques more quickly and efficiently than clinical trials. Only those techniques that are promising need be carried forward to clinical trials. In this way, the temporal bone preparation is a valuable tool for advancing the progress of tympanoplasty surgery.

## SURGICAL IMPLICATIONS FOR TYPE IV TYMPANOPLASTY

A basic assumption made by us is that stapes velocity determines the cochlear response and, in turn, the hearing level.[3,4,9] Therefore, our measurements of stapes velocity can be used as indicators of the functional effect of structural variations in our temporal bone model of type IV tympanoplasty. The variations that produce the largest stapes velocities can be considered to produce the best hearing results. We will focus our discussion on the effect of variations in the acoustic properties (impedances) of the stapes footplate, the acoustic shield, the cavum minor, and the round window.

*Stapes Footplate.* Not surprisingly, our data indicate that an increase in the impedance of the stapes footplate and annular ligament (produced, in our case, by drying) can result in a significant reduction in the mobility of the stapes (Fig 6). Therefore, *the footplate should be kept as mobile as possible.* Prac-

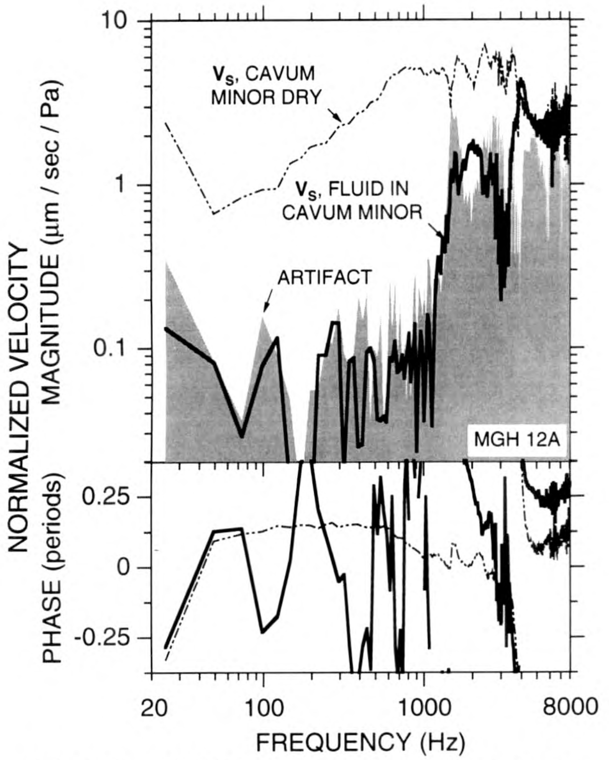

**Fig 8.** (Bone MGH 12A) Change in stapes velocity **Vs** when cavum minor was filled with saline (solid line), mimicking decrease in cavum minor volume *VCM* to zero. All curves are normalized by **Pow**. **|Vs|** is much less than when cavum minor was air-filled (dot-dashed line) and is indistinguishable from mechanical artifact (shaded area).

tically, postoperative deposition of fibrous tissue on the surface of the footplate is likely to lead to partial fixation of the annular ligament and thereby lead to an unsatisfactory hearing result. It is important to prevent this by cleaning the surface of the footplate meticulously and delicately of all granulation tissue and disease. Proliferation of fibrous tissue can be prevented by resurfacing the footplate with a very thin *split-thickness skin graft*, on the order of 0.08 mm (0.003 in) thick, that is carefully invaginated into the oval window niche as advocated by Gotay-Rodriguez and Schuknecht.[2] A temporalis fascia graft should *not* be placed on the footplate, since it is much thicker than a skin graft and may lead to partial fixation of the stapes.

*Acoustic Shield.* Figure 7 demonstrates that the mechanical properties of the acoustic shield play a critical role in determining the window pressure differences and the hearing result after a type IV tympanoplasty. Ideally, in order to maximize the difference in sound pressure between the oval and round windows, *the shield should have as high an impedance as possible*. A large impedance results from a large graft stiffness and mass. As the impedance of the shield diminishes, so will stapes motion; inadequate shielding leads to a low-frequency air-bone gap (despite adequate stapes mobility and good aeration of the cavum minor). Furthermore, our results demonstrate that temporalis fascia, which is a commonly used shield material, may not always lead to an optimal hearing result. Better results may be achieved with more impedant shields made of cartilage or Silastic silicone rubber sheeting. According to our model analyses,[4,9] a sufficiently impedant shield is particularly important when cavum minor aeration is suboptimal. Therefore, we recommend that a fascia shield be stiffened with a piece of cartilage or Silastic silicone rubber. This can be done quite readily by positioning a crescent-shaped piece of cartilage or Silastic silicone rubber (1-mm thickness) on the deep surface of the fascia graft, as described in a previous report.[5] (As discussed below, the conclusions on shield material arrived at from these measurements are more restrictive than our earlier model estimates.[4,5])

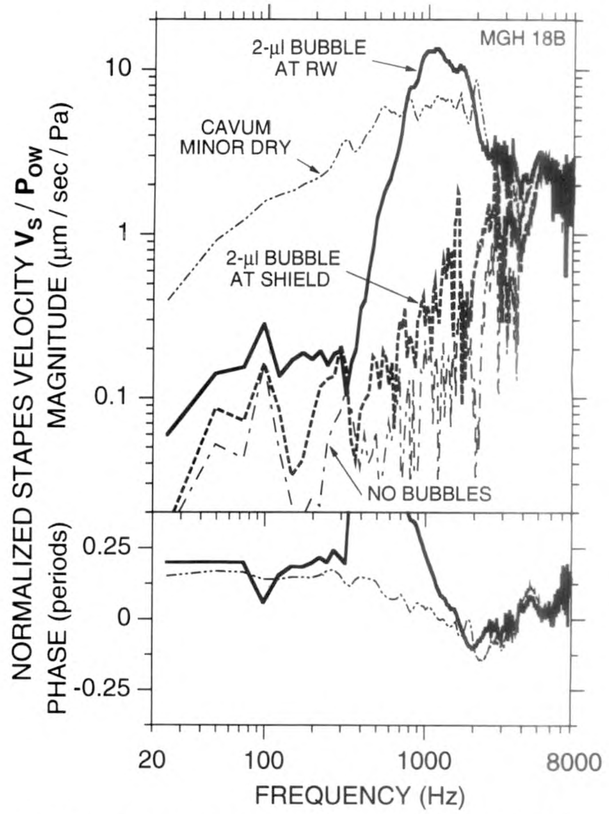

**Fig 9.** (Bone MGH 18B) Effect of air bubble of 2 µL volume on stapes velocity **Vs** when cavum minor was filled with saline. All curves are normalized by **Pow**. When bubble was in contact with round window membrane, **|Vs|** (thick solid line) was comparable to or slightly higher than **|Vs|** with cavum minor dry (double-dot-dashed line) above 700 Hz. At lower frequencies, **|Vs|** with bubble was much smaller than **|Vs|** with cavum minor dry, and **∠Vs** with bubble varied randomly from **∠Vs** with cavum minor dry. When bubble was at shield, **|Vs|** (thick dashed line) was much smaller than when bubble was at round window, and was not much larger than upper bound on **|Vs|** with no bubbles (dot-dashed line). Phase of **Vs** with no bubble in cavum minor or with bubble near shield varied randomly and is not shown.

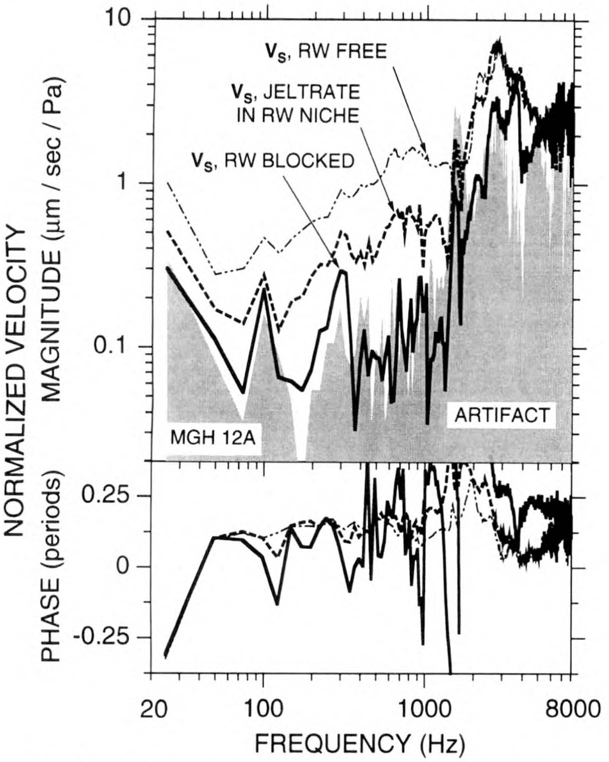

Fig 10. Effect of reducing round window mobility on stapes velocity **Vs** in bone MGH 12A. All curves are normalized by **Pow**. When round window mobility was reduced by filling round window niche with flexible compound, |**Vs**| was reduced by factor of 2 to 3 (thick dashed line) from case when round window mobility was normal (dot-dashed line). When round window was blocked by filling niche with dental cement, |**Vs**| (thick solid line) was indistinguishable from artifact (shaded area).

An issue that is relevant to the above discussion is that in any given patient, the postoperative impedance of a fascia shield also depends on the healing response of the ear. Some fascia shields become thicker due to deposition of fibrous tissue; others become thinner, and some become very thin and atrophic. Thus, it is possible that a shield made of fascia alone could heal such that its impedance is high enough to give an optimal hearing result, but this may not always be the case. Furthermore, our calculations[4,9] suggest that there may not be much of a safety factor in a shield made of fascia alone, in that its impedance may be inadequate when aeration of the cavum minor is suboptimal. In a given patient, if the degree of shielding is suspected to be inadequate, then the shield can be weighted in the office with a crescent-shaped piece of Silastic silicone rubber or with mercury-aquaphor ointment.[19] If such weighting results in an improvement of the air-bone gap, then a revision procedure to stiffen the shield by using cartilage or Silastic silicone rubber could be considered.

*Cavum Minor and Round Window Membrane.* It is well known that adequate aeration of the cavum minor is essential for good hearing results after type IV procedures. Our analysis enables us to quantify the acoustic effects of pathologic alterations of this air space, including accumulation of fluid within the cavum minor, retraction and atelectasis of the acoustic shield, and deposition of fibrous tissue in the round window niche and elsewhere in the cavum minor. Such changes are often encountered clinically and can occur as a result of chronic otitis media or as sequelae of surgery.

*Reductions of the cavum minor air space* can result from retraction or partial atelectasis of the acoustic shield or from deposition of fibrous tissue within the cavum minor in areas other than the round window niche. The anatomic size of a normal cavum minor is about 100 µL.[4] Our data suggest that hearing would not be adversely affected, provided that at least 16 µL of air remains in contact with the round window membrane and that the acoustic shield is adequately stiff. Furthermore, cavum minor volumes of as little as 2 µL appear to be large enough to maintain optimal stapes motion at frequencies above 700 Hz, if the air is in contact with the round window (Fig 9).

A *fluid-filled cavum minor* has an impedance that is much higher than a cavum minor that is filled with air. As a result, fluid filling greatly reduces stapes motion (Fig 8). There is an improvement in stapes motion when an air bubble is introduced into the fluid, but it appears from our results that both the volume and location of the bubble are of importance (Fig 9). An extremely small air bubble, 2 µL in size, located immediately adjacent to the round window membrane is sufficient for near-normal stapes motion above 700 Hz, whereas a 30-µL bubble is needed at points elsewhere in the cavum minor to achieve the same level of stapes motion. (As discussed below, the conclusions on minimum cavum minor volume arrived at from these measurements are less restrictive than our earlier model estimates.[4,5])

*Deposition of fibrous tissue within the round window niche* can occur as part of a healing response to chronic otitis media[20] or as a result of postoperative scarring. The acoustic effects of such fibrosis will vary, depending on the impedance of the deposited tissue and the contents of the remainder of the cavum minor. Our measurements made after filling the round window niche with Jeltrate (Fig 10) suggest that deposition of connective tissue that is sufficiently compliant, in conjunction with an aerated cavum minor, may have little or no adverse effect on hearing. On the other hand, obliteration of the round window niche by thick, dense fibrous tissue could effectively uncouple the round window from the remainder of the cavum minor, and will therefore negate the beneficial effect of an air volume located in the anterior part of the cavum minor (producing, in effect, a fluid-filled cavum minor with an air bubble distant from the round window; Fig 9).

In the clinical setting, it is likely that some patients may demonstrate a combination of pathologic changes within the cavum minor. In such cases, the final acoustic effects of the combinations of retraction or atelectasis of the shield, of fibrosis, and of formation of an effusion within the cavum minor will depend on the interaction of their individual effects.

When performing a type IV tympanoplasty, the goal of the otologic surgeon should be to create conditions that might promote *postoperative aeration of the round window niche and preserve round window mobility*. Every effort should be made to prevent formation of fibrous adhesions between the acoustic shield and medial wall of the hypotympanum and to avoid occlusion of the tympanic orifice of the eustachian tube by fibrous tissue. One should preserve all healthy mucosa lining the round window niche, hypotympanum, and protympanum. If there are areas of exposed bone on the medial wall of the cavum minor, then a crescent-shaped piece of Silastic silicone rubber or Teflon (eg, a Schuknecht crescent) should be placed in the cavum minor to prevent adhesions between the acoustic shield and the medial hypotympanic wall.[2]

## COMPARISON OF MEASUREMENTS TO MODEL PREDICTIONS

The present study provides a means to test specific predictions of our type IV tympanoplasty model *directly*. As was pointed out in the Results section, our experimental measurements closely matched *qualitative* model predictions about the mechanics of the type IV procedure: 1) Changes in the impedance of the acoustic shield produced proportional changes in window pressure difference and stapes velocity, with larger shield impedances producing larger pressure differences and larger velocities. 2) Stiffening the annular ligament resulted in a reduction in stapes velocity. 3) Removing the air in the cavum minor reduced stapes velocity. 4) Immobilizing the round window reduced stapes velocity. However, there were some *quantitative* differences between our data and model predictions, which we will now discuss.

*Impedance of Stapes and Cochlea.* In our model, the value of the stapes and cochlear impedance that was used was that estimated by Kringlebotn.[21] The latter was derived from indirect measurements. Recently, the magnitude of the stapes-cochlear impedance was measured directly and found to be higher than Kringlebotn's estimate by a factor of 3 to 5 (about 10 to 15 dB).[10] In our experimental preparation, measurements of stapes velocity under optimum conditions (a highly impedant shield, a large-enough cavum minor, a freely mobile round window, and a normal stapes and annular ligament, eg, Fig 4) are consistent with the more recent direct measurements of the stapes and cochlear input impedance.[10] While the increased magnitude of the stapes-cochlear impedance has little effect on the qualitative predictions of the model, it has a significant effect on some of its quantitative predictions.

*Minimum Volume of Cavum Minor.* Our experimental measurements and earlier model analysis both describe a minimum effective volume of the cavum minor. In order to achieve optimum results over the entire auditory range, the present measurements suggest that about 20 μL of air is needed near the round window, while the previous analysis suggested a minimum volume of 100 μL.[4] The factor of 5 difference between the two can be attributed to the difference between the measured and the model stapes-cochlear impedance. An optimum cavum minor air volume is one with an impedance that is smaller in magnitude than the impedance of the stapes and cochlea. Since the measured stapes-cochlear impedance is a factor of 5 larger than the model estimate, the real cavum minor volume can be one-fifth the previous minimum estimate.

One result of our measurements that is not at all consistent with the model analysis is our observation of the importance of the *location* of an air bubble in a fluid-filled cavum minor. The new result suggests a need to broaden our model of the cavum minor by including the possibility of a separation of the air space from the round window.

*Minimum Impedance of Acoustic Shield.* Both the data in this report and the earlier model analyses point out that the acoustic shield needs to be of sufficiently high impedance to achieve optimum hearing results. The earlier analyses[4,5] suggested that a shield made of two layers of fascia would be superior to one made of a single layer, and that the two-layer shield might be sufficiently impedant. The present data show that a two-layer shield is not much better than a one-layer shield, and that in both cases, the resulting stapes motion is suboptimal (Fig 7). Indeed, a randomized blinded trial of 12 type IV procedures comparing shields of one versus two layers of temporalis fascia did not show any differences in postoperative hearing results between the two groups (Merchant and Rosowski, unpublished observations).

The earlier quantitative predictions of shield efficacy were based on an incomplete understanding of the mechanical properties of fascia. As noted previously, our estimates of these properties were based on published measurements of the mechanical properties of hyaline cartilage and ligaments.[4,9] We know of no direct measurements of the mechanical properties of temporalis fascia. With the temporal bone preparation, we were able to determine directly the effectiveness of fascia and other shield materials.

## CONCLUSIONS

1. A cadaveric temporal bone preparation was developed to simulate the type IV tympanoplasty procedure. In this preparation, the impedances of the stapes, acoustic shield, cavum minor, and round window were varied individually, and stapes displacement and oval and round window pressures were measured in response to a broadband acoustic stimulus. The largest stapes motions occurred with a mobile stapes and round window, a sufficiently stiff shield, and a well-aerated cavum minor.

2. Acoustic shields made of cartilage or Silastic silicone rubber sheeting 1 mm in thickness produced a near-optimal pressure difference between the oval and round windows and near-maximal stapes motion. Shields made of temporalis fascia produced smaller pressure differences and smaller stapes motion.

3. A cavum minor volume as small as 16 μL was sufficient for optimal stapes motion, and even a 2-μL air space facilitated stapes motion above 700 Hz, provided the air was in contact with the round window membrane.

4. Partial fixation of the stapes footplate or restricting the motion of the round window membrane reduced stapes motion.

5. The results obtained above matched the qualitative predictions of a theoretic model of type IV tympanoplasty that used stapes motion to predict hearing ability.[4,9] There were some quantitative differences, in that the volume of the cavum minor required to preserve stapes motion was smaller than predicted, while the shield needed for optimum results proved to be stiffer than predicted.

6. To optimize the postoperative result, the otologic surgeon should preserve normal stapes mobility, preferably by covering the footplate with a very thin split-thickness skin graft, not a fascia graft; reinforce a fascia shield with cartilage or Silastic silicone rubber 1 mm in thickness; create conditions that promote postoperative aeration of the round window niche; and preserve round window mobility.

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ACKNOWLEDGMENTS — We are grateful to the members of the Eaton-Peabody Laboratory and the Otopathology Laboratory for assistance with this work. Special thanks are due to Diane Jones for obtaining the temporal bones, to Debra S. Louison and Bertrand Delgutte, PhD, for developing the experimental control software, and to Knowles Electronics for donating microphones. Special thanks also go to W. T. Peake, ScD, Joseph B. Nadol, Jr, MD, S. E. Voss, MS, and S. Puria, PhD, for comments on the manuscript.

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*Ann Otol Rhinol Laryngol* 106:1997

# THREE-DIMENSIONAL ANALYSIS OF SPONTANEOUS NYSTAGMUS IN PERIPHERAL VESTIBULAR LESIONS

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The direction of spontaneous nystagmus was recorded in three dimensions with scleral dual search coils in three patients after vestibular neurectomy and in seven patients with vestibular neuritis. The rotation vectors of the spontaneous nystagmus clustered along the sensitivity vector of the lateral semicircular canal (SCC). The direction of the spontaneous nystagmus after resection of the whole eighth nerve was not different from that after resection of only the superior branch of the vestibular nerve. Deviations from this direction were observed only after resection of the inferior vestibular nerve and in one patient with vestibular neuritis. The absence of nystagmus components in direction of the vertical SCC reflects an anisotropy of oculomotor efferents of the vestibulo-ocular reflex arc rather than a lesion limited to the lateral SCC afferents. Therefore, the three-dimensional analysis of spontaneous nystagmus does not permit accurate localization of a peripheral vestibular lesion.

KEY WORDS — eye movements, search coil, semicircular canals, vestibular neurectomy, vestibular neuritis.

## INTRODUCTION

The three pairs of semicircular canals (SCCs) are positioned in three nearly perpendicular planes in the head and act as angular accelerometers. They provide the sensory input for the generation of the vestibulo-ocular reflex (VOR), which stabilizes gaze during angular head movements by rotating the eyes oppositely to the head movements in all directions. A sudden unilateral lesion of the peripheral vestibular system not only eliminates one counterpart of each pair of the vestibular accelerometers, but also abolishes the tonic neuronal discharge (resting activity) in the nerve on that side. The resulting imbalance between the tonic input of the two ears leads to the generation of reflexive eye movements (spontaneous nystagmus). Acute peripheral vestibulopathy ("vestibular neuritis") is the prototype of a sudden unilateral peripheral lesion. The cause of this common disorder is still unclear, but there is good evidence that different parts of the labyrinth may be involved to a variable extent in different patients. The clinical parameters spontaneous nystagmus, caloric canal paresis (reflecting mainly the function of the lateral SCC), and deviation of the subjective visual vertical (a parameter of mainly otolithic function) may be dissociated.[1]

Electrical stimulations of single SCC nerves in animals have shown that the SCC afferents induce eye movements roughly in the corresponding anatomic plane.[2] From this, one would conclude that lesions of single SCCs induce spontaneous nystagmus also in the corresponding canal plane. Clinically, however, spontaneous nystagmus is usually analyzed only in its horizontal (and vertical) component, because of technical limitations. Recent developments[3] permit accurate analysis of the eye movements in three dimensions, ie, in all degrees of freedom, also in human patients. The aim of this study was to investigate whether the recording of the spontaneous nystagmus in three dimensions permits a more accurate localization of lesions of the vestibular end organ.

## MATERIAL AND METHODS

*Patients.* Three-dimensional (3-D) spontaneous nystagmus was recorded in three patients after vestibular neurectomy (patients VN1 through VN3) and in seven patients suffering from acute peripheral vestibulopathy (patients APV1 through APV7). Surgery was performed for relief from incapacitating vertigo due to Meniere's disease. (We thank Prof U. Fisch for permission to perform these measurements in patients operated on by him.) In patient VN1, a translabyrinthine cochleovestibular neurectomy was performed. The internal auditory canal was reached by removing the SCCs. The intrameatal segment of the vestibular nerve, including Scarpa's ganglion, and the most peripheral part of the cochlear nerve were resected.[4] A selective supralabyrinthine vestibular neurectomy was performed in patient VN2, who had only a moderate hearing loss of the affected ear.

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CLINICAL FINDINGS AND COMPONENTS OF NYSTAGMUS IN DESCRIBED PATIENTS

| *Patient* | *Sex* | *Age (y)* | *Side* | *Caloric Canal Paresis (%)** | *Time of Nystagmus Recording (d)†* | *Spontaneous Nystagmus (°/s)* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *x* | *y* | *z* |
| Vestibular neurectomies | | | | | | | | |
| VN1 | M | 40 | R | 43 | 5 | 4.3 | 1.9 | −10.8 |
| VN2 | F | 34 | R | 69 | 2 | 3.6 | 2.7 | −11.1 |
| VN3 | M | 62 | R | 100 | 4 | 2.9 | −1.0 | −3.2 |
| Acute peripheral vestibulopathies | | | | | | | | |
| APV1 | M | 57 | R | 43 | 2 | 5.3 | 2.2 | −15.9 |
| APV2 | F | 35 | R | 60 | 5 | 2.9 | 0.6 | −18.7 |
| APV3 | M | 67 | L | 100 | 6 | −4.3 | 1.8 | 9.3 |
| APV4 | M | 49 | R | 100 | 10 | 3.4 | 2.2 | −17.0 |
| APV5 | M | 73 | L | 100 | 10 | −0.3 | 0.3 | 4.1 |
| APV6 | M | 63 | L | 100 | 10 | −0.1 | 1.7 | 3.8 |
| APV7 | M | 45 | L | 100 | 11 | −2.0 | 6.6 | 9.9 |

Patient VN1 had translabyrinthine cochleovestibular neurectomy, patient VN2 had supralabyrinthine vestibular neurectomy, and patient VN3 had translabyrinthine cochleovestibular neurectomy 16 years after homolateral supralabyrinthine vestibular neurectomy.

*In VN patients, before surgery; in APV patients, after onset of disease.

†In VN patients, days after surgery; in APV patients, days after onset of disease.

In this patient, the superior vestibular nerve was completely resected, including Scarpa's ganglion. Due to the very narrow internal auditory canal, only the posterior part of the inferior vestibular nerve was included in the resection in order to avoid disturbing the blood supply of the cochlear nerve. It is reasonable to expect that the saccular nerve fibers and some of the posterior SCC nerve fibers were, therefore, spared in this patient. Patient VN3 was submitted to a supralabyrinthine vestibular neurectomy (as in patient VN2) 16 years previously. He was completely relieved from vertigo, but still suffered from an incapacitating tinnitus. Therefore, the cochlear nerve was resected through a translabyrinthine approach as in patient VN1. Intraoperatively, a few residual fibers of the singular nerve (innervating the posterior SCC crista) and half the normal numbers of fibers of the ramus sacculi, but no residual fibers of the superior vestibular nerve, were found and removed. The preoperative clinical findings and time of postoperative eye movement recordings are shown in the Table.

Patients APV1 to APV7 experienced a sudden onset of vertigo with spontaneous nystagmus and unilaterally impaired caloric reaction (see Table). Exclusion of a specific ear disease or other neurologic deficits suggested the diagnosis of an acute peripheral vestibulopathy ("vestibular neuritis").

*Three-Dimensional Recording of Eye Movements.* The eye movements were recorded in three dimensions with dual search coils (manufactured by Skalar Instruments, Delft, the Netherlands), which were mounted in a soft contact ring placed on the locally anesthetized right sclera. The patients were sitting upright. The head rested on a chin bar, and was fixated by frontal and occipital blocks inside a coil frame (70 cm diameter) with earth-horizontal and earth-vertical magnetic fields. The measured right eye was exactly in the center of the frame. The angle between the stereotaxic horizontal plane of the head (defined by the outer acoustic meatus and the lower orbital rim) and the earth-horizontal plane was determined in order to translate the recorded eye movements from the earth-fixed recording coordinate system into head stereotaxic directions. Analog raw coil signals were obtained by a Skalar Eye Position Meter Type 3000, digitized with 833 Hz, and analyzed offline.

*Calibration.* The dual search coils were first calibrated "in vitro" on a three-axis gimbal system inside the magnetic frame. Measuring the induced voltages in both coils at well-defined 3-D gimbal positions allowed us to determine the angle between the two coils and their sensitivity vectors. "In vivo" calibrations were performed before each trial by asking the patients to fixate on small dots that were presented on a tangent screen (distance 164 cm) in the center and 20° in the horizontal and vertical directions with respect to the right eye. Measuring the induced search coil voltages at these positions allowed us to compute the instantaneous 3-D orientation of the coils — and of the eye — during subsequent periods of spontaneous eye movements. The precise algorithm of the procedure is described elsewhere.[5]

*Experimental Procedure.* After calibration, the patients were asked to fixate on the center dot on the tangent screen. Then the lights were turned off, and the eye movements were recorded for 10 seconds in complete darkness. During this time the patients were verbally encouraged to keep the current eye position. This procedure was repeated several times for central

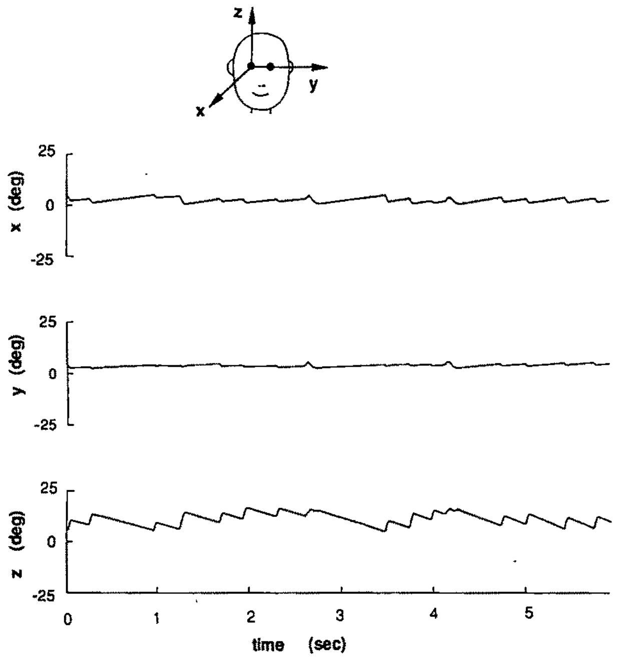

Fig 1. Sample of 6-second period of spontaneous nystagmus (eyes open in darkness, patient VN1). Eye movement components along three orthogonal axes are shown separately. x —rotation axis for torsional movements, y — vertical movements, z — horizontal movements. For signs of direction, see text.

gaze, as well as for gaze positions 20° to the right and 20° to the left, respectively.

*Data Analysis and Presentation.* For each gaze direction, the spontaneous nystagmus was analyzed from representative recording intervals of 6 seconds' duration. First, data points were converted into rotation vectors[6] and rotated from frame coordinates into stereotaxic head coordinates (Fig 1). Then, angular velocity vectors were computed.[7] An angular velocity vector is oriented parallel to the axis about which the eye bulb rotates in reference to a 3-D orthogonal head-fixed coordinate system, in which the torsional axis is parallel to the stereotaxic horizontal plane. Velocities are given in degrees per second. Horizontal eye movements are presented as vectors along the vertical (head) z-axis, vertical eye movements as vectors along the interaural y-axis, and torsional eye movements as vectors along the sagittal x-axis (see scheme at top of Fig 1). According to the right-hand rule, leftward and downward rotations are positive. Extorsional rotations of the upper pole of the right eye and intorsional rotations of the left eye correspond to a positive torsional vector. Figure 2 shows the instantaneous angular velocity rotation vectors during this 6-second period in the sagittal (zx) and the frontal (zy) head planes. From these data, the vectors at each

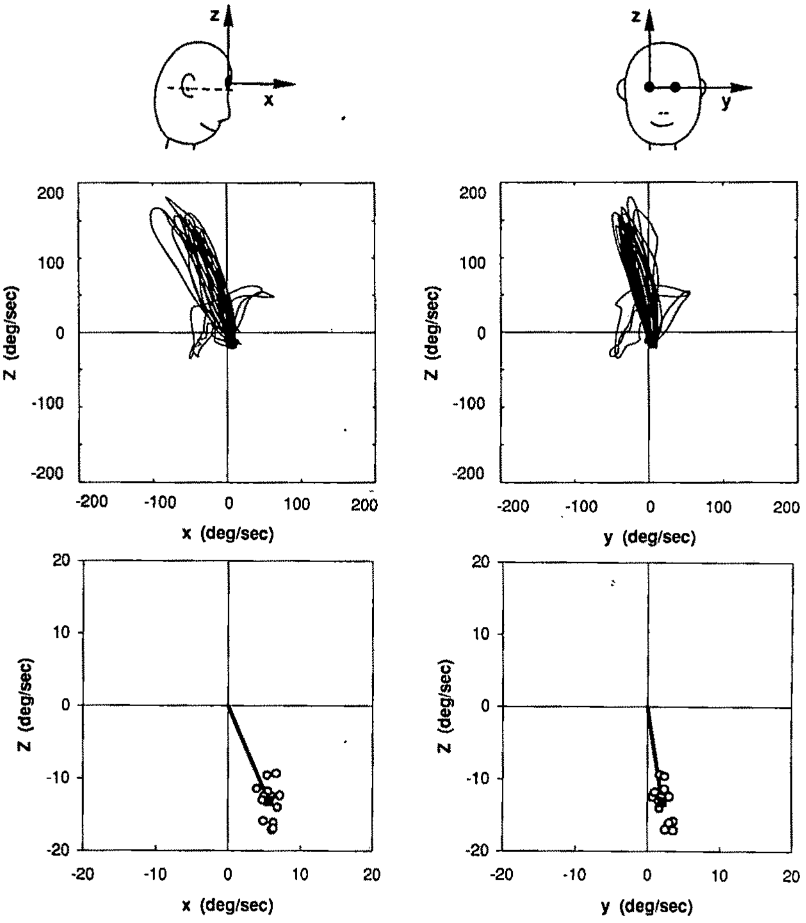

Fig 2. Angular velocity vectors during 6-second period of spontaneous nystagmus shown in Fig 1 in stereotaxic sagittal plane (left) and frontal plane (right). Upper row shows instantaneous vectors. Note vectors with angular velocity vectors around 15°/s (nystagmus fast phases) and oppositely directed vectors with angular velocities around 15°/s (nystagmus slow phases). Direction of these vectors indicates orientation of axis about which eye bulb rotates in reference to head-fixed coordinate system. Slow phase angular velocity vectors of each nystagmus beat (open circles) at instant 120 milliseconds after preceding fast phase and mean of these vectors (solid line and square) are shown in lower row. Note enlarged scale in lower graphs.

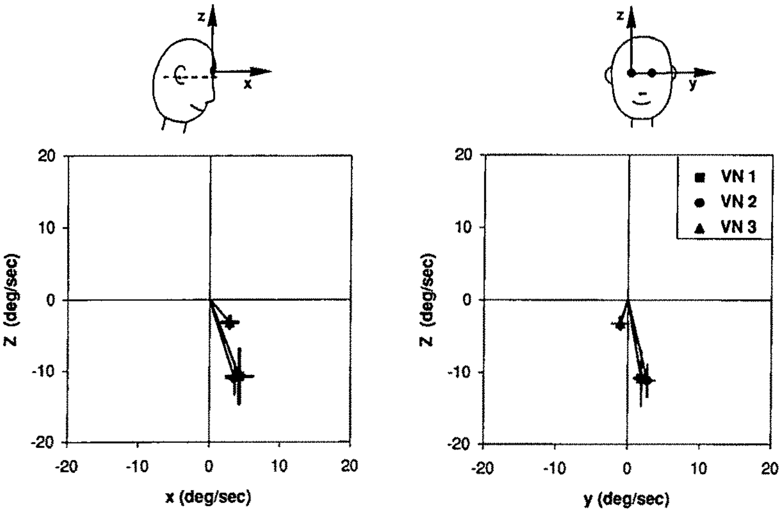

Fig 3. Mean slow phase angular velocity vectors, ±1 SD, of spontaneous nystagmus in three vestibular neurectomized patients. Patient VN1 had translabyrinthine cochleovestibular neurectomy, patient VN2 had supralabyrinthine vestibular neurectomy (superior branch of vestibular nerve), and patient VN3 had resection of inferior branch of vestibular nerve.

instant 120 milliseconds after the time when the vectorial velocity of a quick phase had dropped below 50°/s were selected as representative samples of each nystagmus slow phase (open circles in the lower graphs in Fig 2). The mean of these vectors (solid line and squares) represents the spontaneous nystagmus of a single recording period; several recording periods were averaged for calculating the direction and velocity of the spontaneous nystagmus of each patient.

## RESULTS

*Spontaneous Nystagmus Following Vestibular Neurectomy.* A sample of spontaneous eye movements recorded in three dimensions in darkness in patient VN1 after resection of the (right) eighth nerve is shown in Fig 1. The most prominent components of this spontaneous nystagmus are fast phases to the left (+z) and slow eye movements to the right (–z). There are additional torsional (+x) and downward (+y) slow eye movements with saccades in the opposite direction. In Fig 2 the eye movements during this 6-second period are plotted as angular velocity vectors in the stereotaxic sagittal (xz) and frontal (yz) planes. The plots of the instantaneous angular velocity vectors (upper row) show fast eye movements of >150°/s with a negative x component in the sagittal plane and a negative y component in the frontal plane, as well as eye movements with much lower peak amplitudes (about 15°/s) in the opposite direction. These nystagmus slow phases are shown also as angular velocity vectors of each nystagmus beat in the lower graphs in Fig 2. In the sagittal plane, the mean angular velocity vector (which may be considered as the rotation axis of the eye bulb during the nystagmus slow phase) is tilted by the torsional (+x) eye movement components 23.0° ventrally. In the frontal plane there is a tilt of the mean angular velocity vector of 8.6° laterally to the left by the downward (+y) component.

After selective resection of the (right) superior vestibular nerve (patient VN2), the spontaneous nystagmus did not differ from that observed after total eighth nerve resection with regard to the angular velocity and to the direction of the slow component (Fig 3). Immediately after resection of the (right) inferior division of the vestibular nerve (16 years after removal of the homolateral superior vestibular nerve; patient VN3), a vigorous torsional, oblique left down-beating spontaneous nystagmus was seen with Frenzel glasses. The intensity of this spontaneous nystagmus declined rapidly during the next few days. When the 3-D recordings were performed on the fourth postoperative day, the mean slow phase velocity had declined to 4.7°/s. The mean angular velocity vector was directed more ventrally (relatively larger positive x component) than in patients VN1 and VN2 and was tilted right laterally (negative y component) instead of left laterally (Fig 3), confirming the clinical observation of a positive torsional (ie, extorsion of the upper pole of the right eye and intorsion of the upper pole of the left eye) and downward-beating nystagmus (fast phase).

*Alexander's Law.* The slow phase velocity of the spontaneous nystagmus varied as a function of the direction of gaze. In patient VN2, the horizontal slow phase velocity increased from 11.1°/s when looking straight ahead to 15.4°/s with lateral gaze 20° in the direction of the fast phase. With gaze of 20° in the opposite direction, the horizontal slow phase velocity decreased to 4.3°/s. There was, however, a similar increase and decrease of the eye movement components in the other directions; the direction of the nystagmus slow phase velocity vector thus did *not* change with changes of gaze direction (Fig 4).

*Correlation With Orientation of Semicircular Canals.* Figure 5 compares the direction of the spontaneous nystagmus with the stereotaxic orientation of

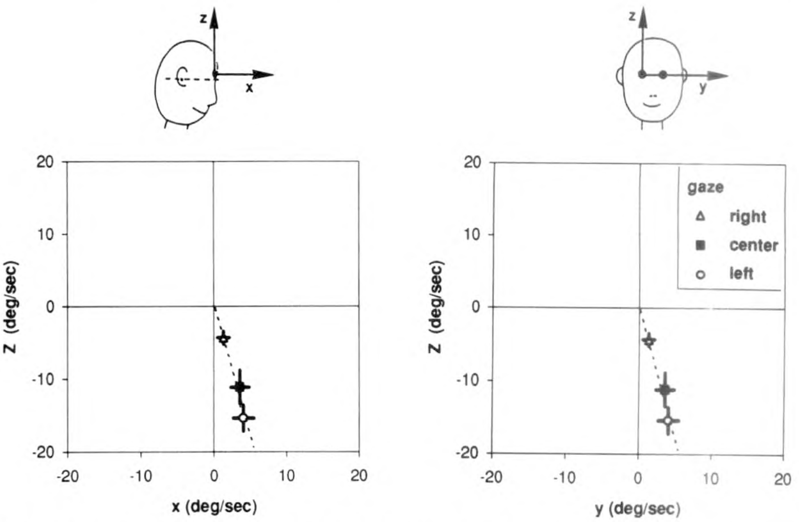

Fig 4. Mean slow phase velocity vectors, ±1 SD, of spontaneous nystagmus as function of direction of gaze (patient VN2). Note increase of angular velocity with gaze toward healthy ear (left) and decrease with gaze toward affected ear (Alexander's law), but no change of direction.

the SCC. The latter data were taken from Blanks et al[8] and are presented as (right) canal plane vectors. Head rotations about an axis parallel to these vectors induce maximal stimulation of a given SCC. The direction of the spontaneous nystagmus in patients VN1 and VN2 is closely collinear with the lateral canal plane vector. After resection of the inferior vestibular nerve (patient VN3), the direction of the nystagmus points in a direction corresponding to a vectorial addition of at least the lateral and the posterior canal vectors. (Only the loss of lateral canal function can induce a negative z component, ie, a left-beating nystagmus, and only the loss of the posterior canal can induce a negative y component, ie, a down-beating nystagmus.)

*Spontaneous Nystagmus in Acute Peripheral Vestibulopathies.* All patients with acute peripheral vestibulopathies had a spontaneous nystagmus with a horizontal slow phase component toward the affected ear (nystagmus beating toward the healthy ear). Within 11 days after onset of the disease, the horizontal slow

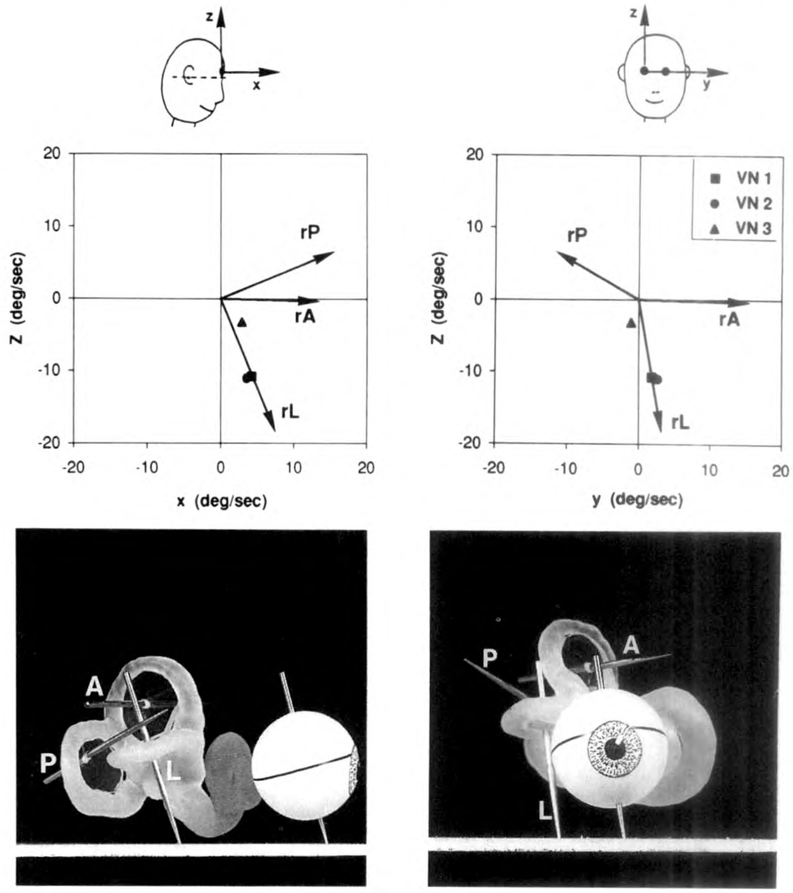

Fig 5. Mean slow phase velocity vectors induced by vestibular neurectomy compared to sensitivity vectors of semicircular canals. rL, rA, and rP indicate vectors of anatomic planes of right lateral, right anterior, and right posterior semicircular canals (data from Blanks et al[8]). These vectors are normalized; in this Figure, their unity value in direction of their maximum response corresponds to 20°/s. Polarity indicates direction of rotations that induce maximal inhibition of corresponding semicircular canal (corresponding to sudden loss of function). At bottom, these semicircular canal plane vectors are shown as bars attached to model of right labyrinth together with nystagmus angular velocity vector shown as rotation axis through model of eye bulb. A indicates right anterior, L right lateral, and P right posterior semicircular canal plane vector. White floor of this model represents stereotaxic horizontal (xy) plane.

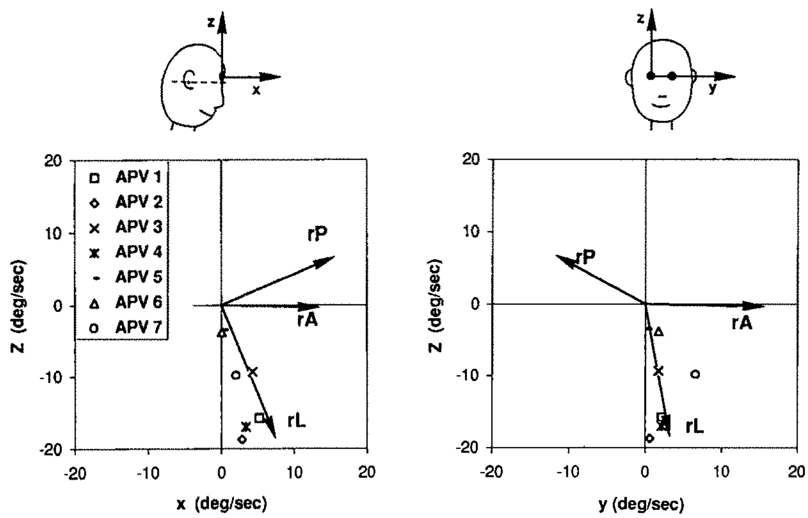

Fig 6. Mean slow phase velocity vectors of spontaneous nystagmus in patients with vestibular neuritis together with sensitivity vectors of (right) semicircular canals. All patients are treated as if right ear was affected. Note alignment of vectors along lateral semicircular canal plane vector with exception of patient APV7. For abbreviations, see Fig 5.

phase velocity was between 9°/s and 19°/s in five patients and 4.1°/s and 3.8°/s in patients APV5 and APV6, respectively. For better comparison, the directions of the nystagmus components were reversed in the patients with left-sided disorders, as if the right ear had been affected. The direction of the mean angular velocity vectors closely coincided with the lateral canal vector in all but one patient (Fig 6). The latter patient (APV7) had a relatively stronger positive y component (slow phase down) of his spontaneous nystagmus when recorded 11 days after onset of the disease.

## DISCUSSION

Clinicians typically describe a spontaneous nystagmus as movements of the pupil relative to the orbit with horizontal, vertical, and torsional components. However, slow phase eye movements are generally produced by angular rotation of the eye bulb about a *single, head-fixed axis* running through the center of the eye *bulb*.[9] This axis may change over time, but — as shown in this study — its orientation in the head is relatively stable in spontaneous nystagmus after peripheral vestibular lesions and, furthermore, is independent of gaze position. A change of gaze position means a movement of the pupil to another place in the orbit. With a spontaneous nystagmus about a head-fixed axis, the pupil will thus perform different movements, depending on the direction of gaze. Presume, for example, rotations of the eye bulb about an axis parallel to the vector of the posterior SCC, which runs in an approximately horizontal head plane some 45° antero-ipsilaterally to the sagittal head plane. During ipsilateral gaze, the axis of gaze will be nearly parallel to the axis of eye rotation and the pupil will perform essentially torsional movements. When the gaze is directed, however, to the contralateral side, the axis of gaze will be orthogonal to the axis of rotation and the pupil will perform essentially vertical movements in the orbit. Conventional electro-oculographic recording techniques mimic this gaze dependency,[10] because they record the horizontal and vertical movement of the electrical axis of the eye bulb (coinciding with the gaze axis) relative to the electrodes attached around the orbit, while torsional movements (along the axis of gaze, ie, nearly parallel to the electrical axis of the eye bulb) are completely missed by this technique. The 3-D search coil technique used in this study, on the other hand, allows for accurate determination of the rotation axis of the eye bulb independent of the direction of gaze. The analysis of Alexander's law has shown that the rotation axis of the spontaneous nystagmus induced by vestibular neurectomy does not change with different horizontal gaze directions.

This provides the means to investigate how (or whether) an underlying vestibular lesion influences the direction of spontaneous nystagmus. Each SCC seems to induce eye movements that are roughly in the direction of its anatomic plane.[2] On physiological stimulation, the three pairs of SCCs, which are oriented in three nearly orthogonal planes in the head, generate compensatory eye movements (VORs) approximately in the direction of the head movement. This suggests vectorial addition of the sensory information originating from the six SCCs. The spontaneous nystagmus in peripheral vestibular disorders is attributed to the loss of the neuronal resting activity in the diseased vestibular afferents. One therefore may hypothesize that the angular rotation axis of spontaneous nystagmus could reflect the vectorial addition of the remaining SCC afferents.

A vestibular neurectomy represents a relatively well-controlled lesion that permits the evaluation of this hypothesis. Following deafferention of all three SCCs (cochleovestibular neurectomy), the eye movement vector coincided with the lateral SCC vector.

Theoretically, one would have expected a larger positive x (torsional) component induced by the simultaneous loss of the resting activity of the anterior and posterior SCC afferents. The vectors of these two SCC have similar x (torsional) directions, but oppositely directed y (vertical) components (Fig 5). Following deafferentation of the lateral and anterior SCCs and of the utricle (supralabyrinthine selective vestibular neurectomy), the angular velocity vector of the induced nystagmus also coincided with the lateral SCC vector. No significantly stronger positive y component (vertical downward slow component) was seen as one would expect from a vectorial addition of the loss of the lateral and anterior SCC afferent activity. Some effect of a sudden deafferentation of the posterior SCC, however, was seen after resection of the residual fibers of the inferior vestibular nerve in patient VN3 (cochleovestibular neurectomy following selective vestibular neurectomy). In this case, the relatively larger positive x component and the negative y component look like a vectorial addition resulting from a loss of the posterior and lateral SCCs, although the lateral SCC had been already deafferentated at the first operation 16 years earlier.

The complete absence of caloric responses in the diseased ear in five of the patients with acute peripheral vestibulopathies suggests a severe lesion of the labyrinth, which is very unlikely to be limited to the lateral SCC. Nevertheless, the angular velocity vector of the spontaneous nystagmus in these patients coincided closely with the lateral SCC vector (or had an even smaller x component). As in the patients with vestibular neurectomies, no nystagmus components in the directions of the vertical canals could be detected, with the exception of the patient with the largest delay between onset of disease and time of recording (APV7). A different organization of the horizontal, vertical, and torsional VORs is probably the reason for these unexpected findings. The six SCCs only differ in respect to the direction of maximal sensitivity to angular acceleration (a cosine function of the angle between the plane of the SCC and the rotation axis of the stimulus). Otherwise, all the SCCs have very similar physiological properties.[11] This suggests that a rotation about any axis gives rise to an equally weighted activity in the brain stem, ie, to an isotropic vestibular input. The oculomotor output of vestibular stimulation, on the other hand, is anisotropic; ie, the horizontal VORs differ from the vertical and torsional VORs in regard to gain (maximal slow phase velocity) and time constant. This anistropy of the oculomotor output even leads to a disalignment of the direction of reflexive eye movements from the direction of head rotation when the latter occur about an oblique axis.[12] Furthermore, the velocity storage mechanism in the brain stem, which prolongs the time constant of the vestibular signals, introduces another anisotropy by enhancing predominantly the horizontal eye movements.[13] This may also tilt the eye angular velocity vector of spontaneous nystagmus toward the lateral SCC vector. Therefore, estimating the contribution of each SCC to the generation of spontaneous nystagmus by mere vectorial addition is insufficient and an oversimplification. Furthermore, additional effects of utricular and saccular lesions may have to be considered. These are even more difficult to assess, because these organs do not have uniform sensitivity vectors, and therefore no distinct directions of eye movements can be attributed to their stimulation or loss of function.

In conclusion, the 3-D angular velocity vectors of the spontaneous nystagmus induced by vestibular neurectomy and in acute peripheral vestibulopathy were found to be surprisingly strongly clustered along the sensitivity vector of the lateral SCC. Significant deviations from this direction were only observed after resection of the inferior branch of the vestibular nerve and in one out of seven patients with acute peripheral vestibulopathy. The absence of nystagmus components in directions of the vertical SCCs may reflect an anisotropy of the oculomotor efferent part of the VOR arc rather than lesions limited to the lateral SCC afferents. The 3-D analysis of spontaneous nystagmus therefore does not permit accurate localization of a peripheral vestibular lesion. Supplementary dynamic stimulation, ie, analysis of VORs in response to angular accelerations in various directions, may give further information in this regard.

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## TWENTY-FOURTH NES CONGRESS

The Twenty-Fourth Ordinary Congress of the International Neurootological and Equilibriometric Society Reg. (NES) will be held April 6-10, 1997, in Haifa, Israel. For information, contact NES Congress President Prof Dr Ludwig Podoshin, Otolaryngology, Head and Neck Surgery, Bnai Zion Medical Center, PO Box 4940, Haifa 31048, Israel; telephone 972-4-359544; fax 972-4-371393.

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## NEUROMONITORING IN OTOLARYNGOLOGY AND SKULL BASE SURGERY

Neuromonitoring in Otolaryngology and Skull Base Surgery will be held in London, England, February 21, 1997. For information, contact Dianne Rooksby, Centre for Continuing Professional Development, University of Nottingham, University Park, Nottingham, NG7 2RD, United Kingdom; telephone 44 115 951 3763; fax 44 115 951 3722; Email dianne.rooksby@nottingham.ac.uk.

*Ann Otol Rhinol Laryngol* 106:1997

# IMMUNOHISTOCHEMICAL LOCALIZATION OF MANGANESE SUPEROXIDE DISMUTASE IN RAT VESTIBULAR DARK CELL REGIONS

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**A modified immunoglobulin peroxidase bridge sequence method was used to detect the localization of manganese superoxide dismutase (MnSOD), a superoxide radical ($O2^-$) scavenging enzyme locating in mitochondrial matrix, in the vestibular labyrinth of pigmented rats. Strong positive MnSOD immunostaining was demonstrated in the dark cell regions of the ampullae, utricle, and common crus. The result provides for the first time direct evidence demonstrating the existence of mitochondrial $O2^-$ scavengers in the vestibular labyrinth and illustrates that the specific sites for vestibular MnSOD immunolocalization are the dark cell regions. This site specificity of MnSOD immunolocalization suggests that dark cell regions may possess high metabolic activity and may encounter constant threat from $O2^-$. We assume MnSOD is needed in protecting some physiologic functions of the dark cell regions. Cell types showing negative MnSOD immunostaining may conceivably be relatively vulnerable to acute $O2^-$ damage.**

**KEY WORDS — manganese superoxide dismutase, superoxide radical, vestibular dark cell region.**

## INTRODUCTION

The generation of intracellular superoxide radicals ($O2^-$) by monovalent metabolic reduction of oxygen appears to arise primarily from electrons escaping from the mitochondrial electron transport chain.[1-3] The intrinsic reactivity of $O2^-$ and its ability to generate the other reactive oxygen species (ROS; hydroxyradical, hydrogen peroxide, and singlet oxygen) constitute a potential threat to cellular integrity.[4,5] Superoxide dismutases (SODs), which can scavenge the $O2^-$, play an important role in the initial cellular defense against these cytotoxic ROS.[3,6-8] In eukaryocytes, two types of SODs can be found, one containing copper and zinc (CuZnSOD), and the other, manganese (MnSOD).[9-11] The CuZnSOD distributes uniformly throughout the nuclear and cytoplasmic matrix, whereas MnSOD exists predominantly in mitochondrial matrix.[10]

To date, there has been increasing interest in the possible involvement of $O2^-$ and their scavengers in the pathophysiology of some diseases.[12-14] Identifying the existence and normal distribution of $O2^-$ scavenging enzymes in the inner ear will be essential for further investigating what roles $O2^-$ and its scavenging enzymes play in the inner ear pathophysiology. Although cochlear SOD activities have been detected,[15,16] it seems difficult to detect SOD activities from the vestibule. A modified immunoglobulin peroxidase bridge sequence method has been shown useful in detecting SODs from various paraffin-embedded rat tissues.[17-19] This technique was also proved to be an effective probe for detecting the specific immunolocalization of mitochondrial MnSOD in paraffin-embedded rat vestibular labyrinth in our study.

Since the mitochondria are significant intracellular sources of $O2^-$ and hence important targets of oxidant injury,[1,2,8] and since the cytosolic CuZnSOD does not appear to be able to cross the mitochondrial membrane[20] to aid in scavenging the $O2^-$ in mitochondria, MnSOD seems to be the one responsible for protecting mitochondria from $O2^-$ damage. The existence of MnSOD is thus considered to be indirect evidence indicating the appearance of $O2^-$, whether generated physiologically or pathologically, in the mitochondria. The inducibility of MnSOD[8,13,21-24] even makes it a useful marker indicating the changes of surrounding environmental factors that influence cellular metabolism. Basic investigations on physiologic MnSOD distribution provide an important database for further research on its pathologic changes.

## MATERIALS AND METHODS

*Animals and Tissue Preparation.* Six healthy 11-week-old male Sprague-Dawley rats (purchased from Japan SLC Inc, Shizuoka, Japan) with a body weight of 250 ± 20 g and strong bilateral Preyer's reflexes were used for the study. All experiments were performed according to National Institutes of Health

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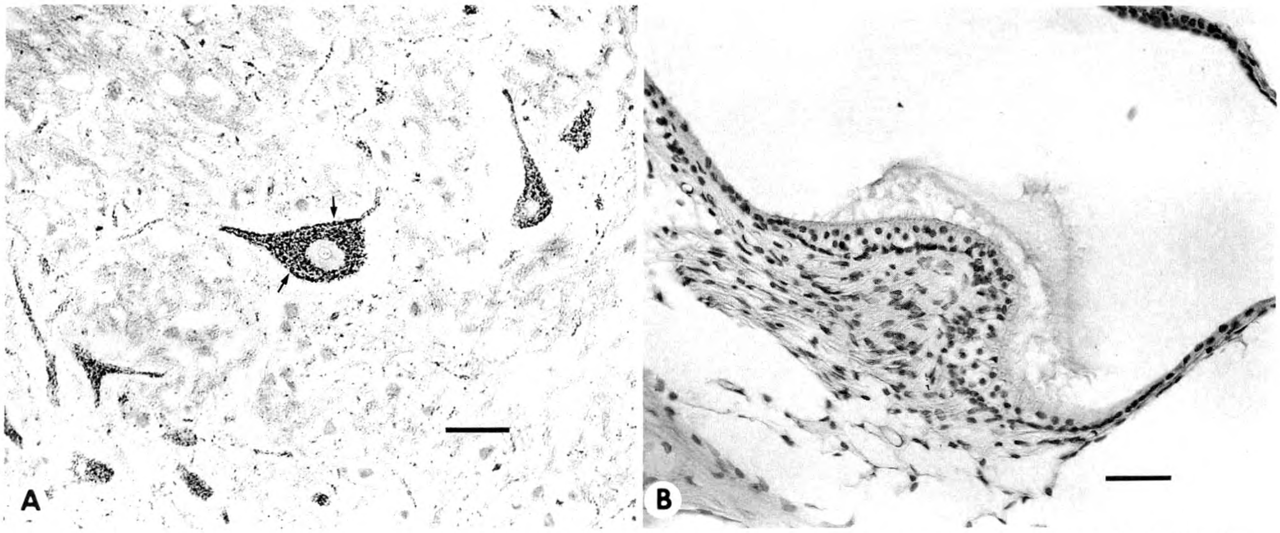

**Fig 1. Controls for manganese superoxide dismutase (MnSOD) immunoreaction (hematoxylin as counterstain). Calibration bars — 30 μm. A) Positive control can be well visualized in cerebral pyramidal cells (arrows) as intracytoplasmic granules. Nucleus clearly shows no positive MnSOD immunostaining. Note that reduced background staining aids in enabling higher resolution of positive immunostaining. B) Negative control shows no granular immunostaining in ampulla.**

(NIH) guidelines on the care and use of laboratory animals and were approved by the institutional committee pertaining to the care and use of animals.

The animals were anesthetized with intraperitoneal pentobarbital sodium (Nembutal, 30 mg/kg) and exsanguinated by intracardiac perfusion with warm lactated Ringer's solution (400 mL/kg body weight), followed by 10% buffered paraformaldehyde (in 0.1 mol/L phosphate buffer solution [PBS], pH 7.2). After cervical decapitation, each crista ampullaris was opened rapidly. The temporal bones were immediately dissected and immersed in the same fixative at 4°C for 24 hours.

After washing with physiologic saline to flush out the fixative, decalcification was performed in a solution containing 0.1 mol/L ethylenediaminetetraacetic acid (EDTA) and 1 mol/L Tris (hydroxymethyl) aminomethane (pH 7.2) at 30°C for 2 weeks. The EDTA solution was changed every other day. The tissues were subsequently dehydrated through a graded series of alcohol preparations, cleared in xylenes, and embedded in paraffin. Serial sections were cut at 4-μm thickness through the vertical cochlear midmodiolar plane. One of every three continuous sections was stained by the immunohistochemical method described by Dobashi et al.[17]

*Immunostaining*. The well-specified rabbit anti rat MnSOD antisera[25] used in this study were kindly provided by Dr K. Asayama (Department of Pediatrics, Yamanashi Medical College, Yamanashi, Japan).

Selected sections were deparaffinized, rehydrated in a graded series of alcohol preparations, and rinsed in 0.1 mol/L PBS. To inactivate endogenous peroxidase, the sections were treated with 3% hydrogen peroxide in methanol for 30 minutes at room temperature. After washing with PBS for 15 minutes, the avidin-biotin peroxidase complex (ABC) method was performed as follows.

Sections were exposed to 10% normal goat serum in 0.1 mol/L PBS for 30 minutes at room temperature to block nonspecific binding. Then the sections were incubated in rabbit anti rat MnSOD antisera diluted in 0.1 mol/L PBS (1:3,000) at 4°C overnight. Rinsed with three changes of 0.1 mol/L PBS for 30 minutes, the sections were then incubated in 1% goat anti rabbit IgG F(ab) fraction labeled with horseradish peroxidase (Vectastain ABC Kit from Vector Laboratories, Inc, Burlingame, Calif) at 37°C for 60 minutes. Rinsed with three changes of PBS for 30 minutes, the sections were then incubated in ABC solution (avidin:biotin:PBS = 1:1:100) of a Vectastain ABC Kit (Vector Laboratories, Inc) for more than 1 hour at 37°C. All the incubations were performed in a wet box. After rinsing with three changes of PBS for 30 minutes, bound primary antibodies were visualized by placing sections in PBS-buffered 0.56 mmol/L 3,3'-diaminobenzidine (DAB; MBL Co Ltd, Nagoya, Japan) containing 1.47 mmol/L hydrogen peroxide substrate medium for 7 minutes. As the intensity of the immunostaining can also be influenced by the time developing in DAB, we precisely controlled the DAB developing time to be the same during each repeated experiment. Some sections were counterstained with hematoxylin after the immunostaining for identifying the cell types.

A positive control reaction for MnSOD immuno-

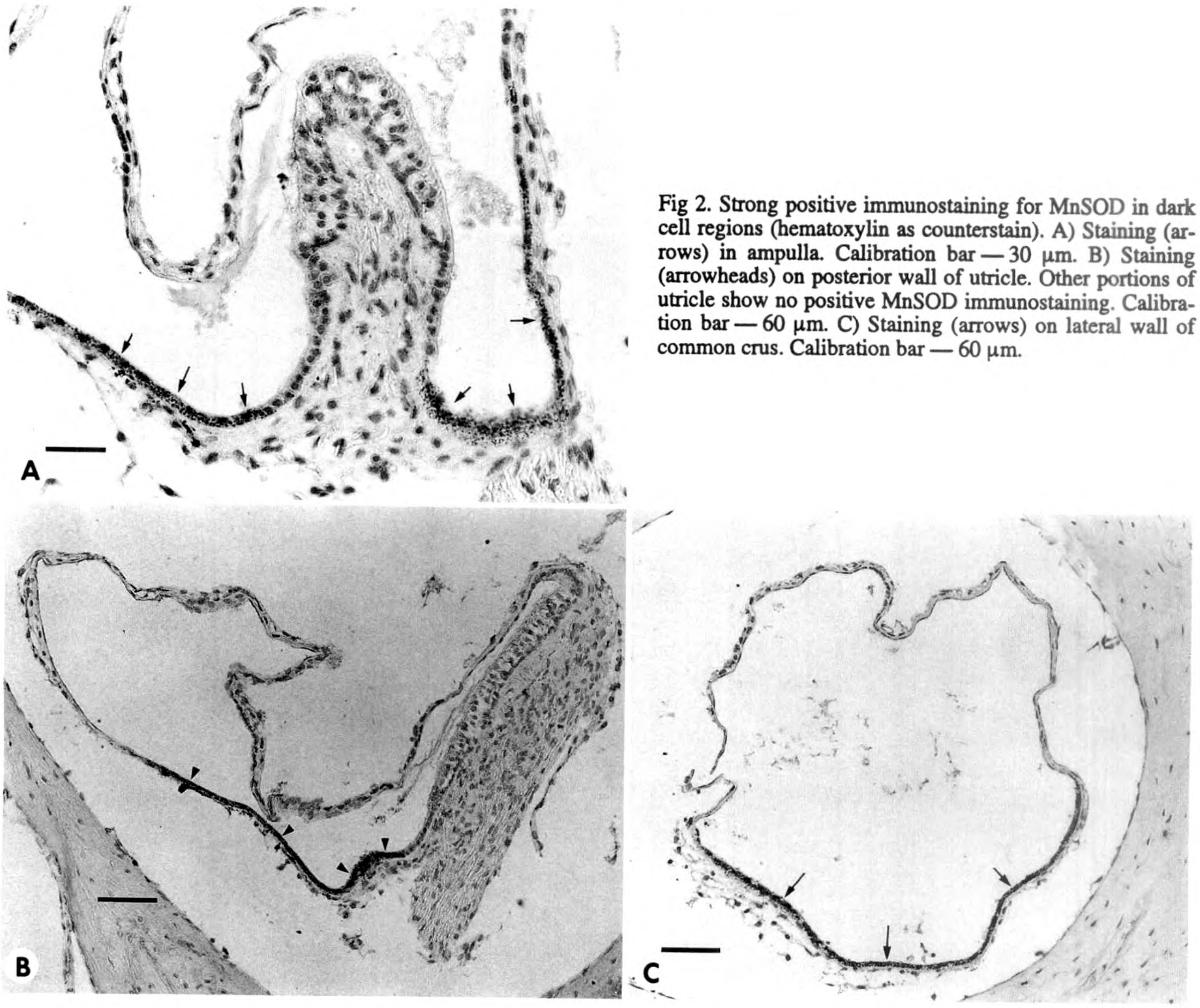

Fig 2. Strong positive immunostaining for MnSOD in dark cell regions (hematoxylin as counterstain). A) Staining (arrows) in ampulla. Calibration bar — 30 μm. B) Staining (arrowheads) on posterior wall of utricle. Other portions of utricle show no positive MnSOD immunostaining. Calibration bar — 60 μm. C) Staining (arrows) on lateral wall of common crus. Calibration bar — 60 μm.

staining was obtained from the cerebral pyramidal cells and was compared to the immunostaining result of the same cells reported by Dobashi et al.[17] For the negative control immunoreaction, nonimmunized rabbit serum at a similar dilution was used instead of the antiserum. To confirm the specificity of the immunostaining, purified MnSOD[25] (100 μg/mL) was used to absorb the anti-MnSOD antibodies (1:3,000 dilution). One repeated experiment was performed to ensure reproducible, accurate results.

## RESULTS

Positive control for MnSOD immunostaining was well demonstrated as bright brownish granules in the cytoplasm of cerebral pyramidal cells (Fig 1A), comparable to those in the report of Dobashi et al.[17] The result of the negative control reaction showed no MnSOD immunostaining (Fig 1B). A specificity test showed results (data not shown) similar to those of the negative control reaction. A false-positive reaction, usually caused by endogenous biotin,[26] had been well blocked by the fixative. Strong positive MnSOD immunostaining was clearly seen in the dark cell regions found on the base of the ampullar crista (Fig 2A), the posterior wall of the utricle (Fig 2B), and the lateral wall of the common crus (Fig 2C). The other regions of the vestibular labyrinth showed no positive MnSOD immunostaining.

## DISCUSSION

Superoxide dismutases are soluble enzymes and easily redistribute artifactually during tissue processing, unless proper tissue fixation is performed immediately. Besides movement caused by poor fixation, diffusional movement of the antigen can also occur during the preparation of paraffin sections and thus result in nonspecific immunostaining. In the current study we used 10% buffered paraformaldehyde to obtain proper tissue fixation and to preserve the MnSOD antigenicity in the rat vestibular labyrinth successfully. Confusing background staining, usually seen in the immunohistochemical stain, did occur in our experiment initially. By using hydrogen peroxide solution to block the endogenous peroxidase activity, diluting the antiserum to ×3,000, and carefully washing between each step, we effectively reduced the background and enabled a higher resolution of positive immunostaining.

It was interesting to notice that strong positive MnSOD immunostaining in the vestibular labyrinth could only be seen in the dark cell regions. The strong positive immunostaining in the dark cell regions is reasonably thought to be due to the abundant mitochondria in which MnSOD exists. But this significant disparity of MnSOD immunostaining found in the vestibular labyrinth may also indicate that different portions of the vestibular labyrinth encounter different surrounding microenvironmental conditions, or that different portions of the vestibular labyrinth have different metabolic functions. Although the whole vestibular labyrinth faces the same vestibular endolymph, only dark cell regions showed intense MnSOD immunostaining. This special immunolocalization suggests that factors causing cellular demand for mitochondrial O2$^-$ scavengers should exist in or come from cells of the dark cell regions or beneath dark cell regions — not from the vestibular endolymph. In the vestibular labyrinth of pigmented animals, melanin-containing melanocytes are known to locate among the interdigitations of the basal infolding of the dark cells.[27] Melanin itself is a homopolymer of indole-5,6-quinone units able to bind and release metal ions and therefore possesses powerful ability in producing functional free radicals.[28,29] Whether there is a relationship between the generation of O2$^-$ from melanocytes, and hence much mitochondrial MnSOD in the dark cell regions, needs further investigation.

Other explanations for this specific immunolocalization of MnSOD were made on the basis of metabolic specialization, including metabolic O2$^-$ generation and the ion pump activity of the dark cells. As the function of MnSOD is to scavenge O2$^-$, strong positive MnSOD immunostaining found only in the dark cell regions certainly suggests that these regions face a more constant threat from O2$^-$ than other portions of the vestibule do. This may further suggest that the dark cell regions probably are the main sites or are close to the sites of vestibular O2$^-$ generation. It has been previously estimated that about 1% to 2% of all oxygen consumed by the mitochondria leaks as O2$^-$,[30] and metabolic changes that require increased dependence on aerobic metabolism will also cause increased synthesis of SOD.[18,31] Increased oxygen consumption with increased mitochondrial O2$^-$ production within the vestibular labyrinth could thus be postulated to occur mainly in the dark cell regions. Accordingly, we can assume that dark cell regions may be highly responsible for the vestibular metabolic activity.

Both O2$^-$ and its derivatives have been identified as able to cause damage to the ion pump activity of the ocular lens.[32] It has also been noticed that a large amount of MnSOD appears to protect the ion pump and the cell itself from O2$^-$ damage.[33] Consequently, the existence of MnSOD in the dark cell regions may also imply the need of O2$^-$ scavengers in protecting the physiologic functions of dark cell regions, since the vestibular dark cells are believed to play an important role in maintaining the homeostasis of the special ion components within the vestibular endolymph[34] by their ion pump activities.[35-37] Furthermore, O2$^-$ can, if metabolically produced in the cell, contribute to ionic gradients, since its small size allows its passage through membranes via anion channels.[38] In order to maintain the stability of the environmental ionic gradients, O2$^-$ scavengers should especially be needed at sites in which O2$^-$ is excessively generated. Thus, MnSOD can be considered an indicator illustrating sites of possible excessive O2$^-$ generation and, at the same time, provide protection against O2$^-$ damage. In this content, the lack of positive MnSOD immunostaining found in the sensory epithelial cells and their supporting cells may suggest the vulnerability of these cell types to acute O2$^-$ damage.

In conclusion, the results of this present study provided direct evidence of the existence of mitochondrial O2$^-$ scavenging enzymes in the vestibular labyrinth and illustrate the specific sites for vestibular MnSOD immunolocalization to be the dark cell regions. This site specificity further suggests that 1) dark cell regions may possess higher metabolic activity relative to other regions of the vestibular labyrinth, 2) dark cell regions may constantly encounter a threat from O2$^-$ or may be responsible for the generation of vestibular O2$^-$, 3) MnSOD may be needed in protecting some physiologic functions of the dark cell regions, and 4) cell types showing no positive MnSOD immunostaining may be relatively vulnerable to acute O2$^-$ damage.

Specific sites for MnSOD immunolocalization in the vestibular labyrinth have been identified in the current study, but since the small molecular size of O2$^-$ enables it to cross cell membranes[38] and diffuse throughout the vestibular system, investigation for the localization of extracellular SOD and cytosolic CuZnSOD will be necessary before the data on vestibular O2$^-$ scavenging enzymes can be fully reconciled.

ACKNOWLEDGMENTS — The authors thank Kohtaro Asayama for providing the antisera and John Rutka for reviewing the manuscript.

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## FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

The Fourth Annual Golf Seminar — Advances in Otolaryngology–Head and Neck Surgery will be held March 11-16, 1997, at the Pinehurst Resort & Country Club in North Carolina. For information, contact Leslie Sullivan, Coordinator, University of Washington School of Medicine Virginia Merrill Bloedel Hearing Research Center, Box 357923, Seattle, WA 98195-7923; E-mail sullivan@u.washington.edu; telephone (206) 685-2962; fax (206) 616-1828.

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## TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

*Ann Otol Rhinol Laryngol* 106:1997

# IMAGING CASE STUDY OF THE MONTH

# JUVENILE CEMENTO-OSSIFYING FIBROMA OF THE MAXILLA

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Juvenile ossifying fibroma is a relatively rare entity within the spectrum of fibro-osseous jaw lesions. We present a case of a teenage girl with maxillary juvenile ossifying fibroma and discuss the radiographic appearance and differential diagnosis.

## CASE REPORT

A 14-year-old girl presented with a 3-month history of bulging right cheek and right nasal obstruction. The patient denied any pain, diplopia, tooth loosening, or bite change. On examination the cheek appeared moderately swollen and firm to palpation. Except for minimal right exophthalmos, the rest of the ophthalmologic evaluation was normal. Rhinoscopy revealed a right nasal cavity obstructed by a medially displaced lateral wall and mucopurulent discharge. The nasal septum was deflected to the left. Findings on the oral and dental examinations were normal. The rest of the physical examination findings were normal.

Plain sinus radiographs showed complete opacification of the right maxillary sinus by a homogeneous-appearing mass extending medially into the right nasal cavity and laterally obscuring the contour of the maxillary bone. Computed tomography (CT) showed a well-demarcated, 5 × 5 × 4-cm round mass that completely filled and expanded the right maxillary sinus (Fig 1). The mass had a relatively high density (higher than muscle) and was fairly homogeneous in appearance, although it contained several areas of low density and scattered calcifications. It appeared to originate from the alveolar ridge (Fig 1B). The medial, lateral, and posterior walls of the sinus were destroyed by the tumor, while the orbital floor was thinned and upwardly displaced. The tumor extended into the right nasal cavity. A magnetic resonance

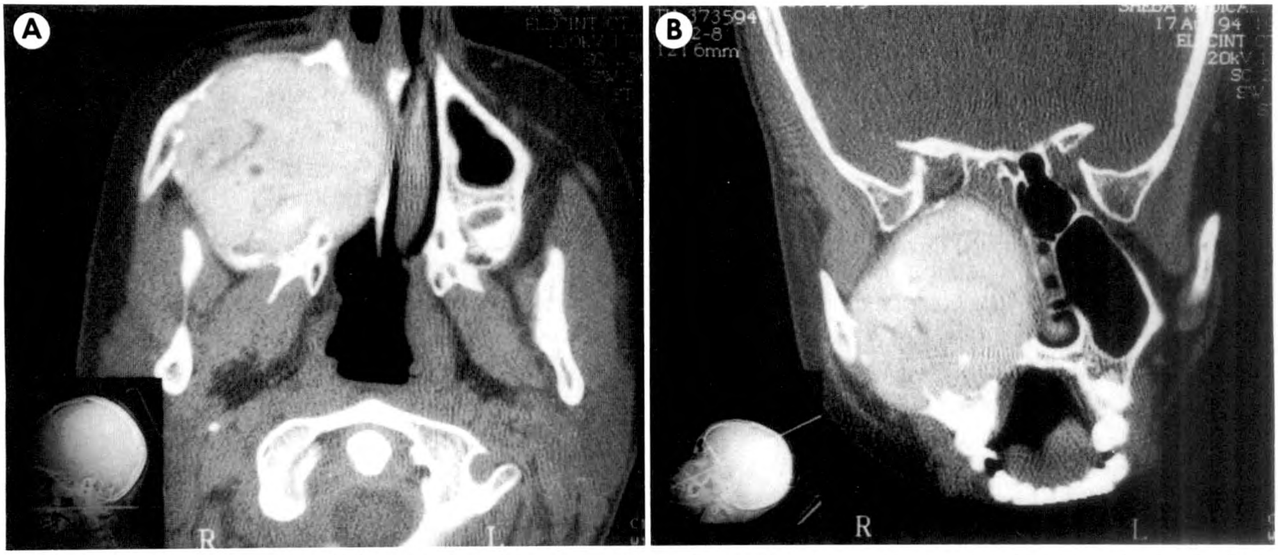

Fig 1. Computed tomograms. A) Axial section. Round mass fills and expands right maxillary sinus, with partial destruction of its walls and extension into nasal cavity. B) Coronal section showing tumor originating from alveolar ridge. On both sections, note scattered low-density areas within tumor, implying cystic changes as well as scattered calcifications.

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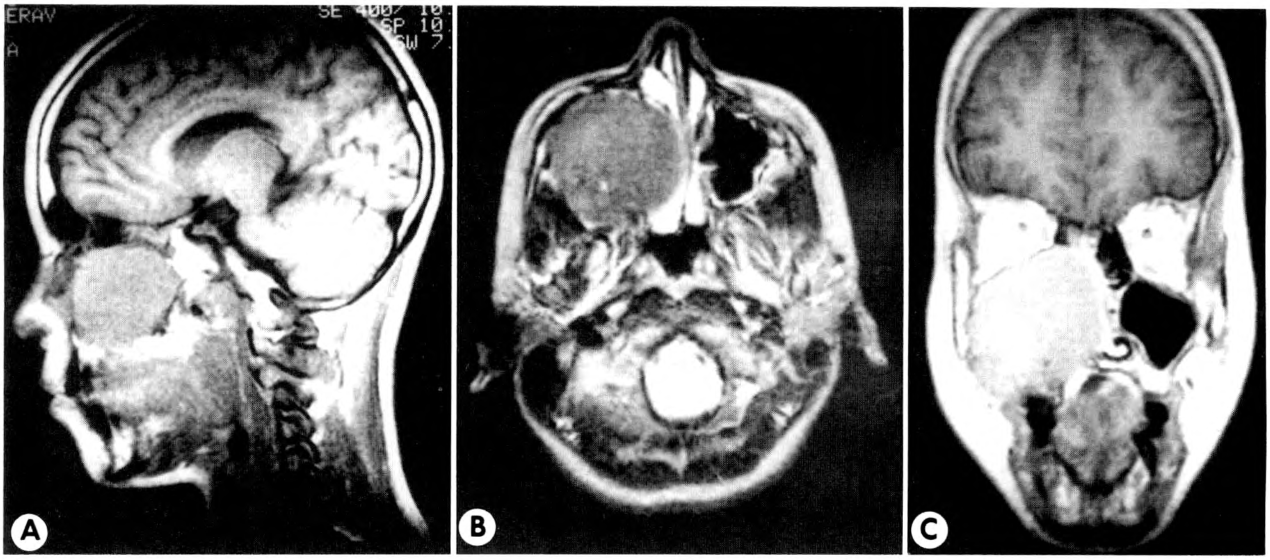

Fig 2. Magnetic resonance images. A) Sagittal T1-weighted image (400/10/1). B) Axial T2-weighted image (1,800/90/1). Tumor is isointense to muscle on both T1 and T2. Small cystic areas are evident as foci of low-T1, high-T2 signal intensities. C) Postgadolinium coronal T1-weighted image (400/10/1) demonstrating mild homogeneous enhancement. Note orbital fat separating tumor from inferior rectus muscle, as well as smaller cross section of right orbit compared to left.

imaging (MRI) study, which included T1-weighted, T2-weighted, and postgadolinium T1-weighted images, demonstrated the mass within the right maxillary sinus (Fig 2). The extent of the mass was demonstrated also on the MRI, as well as the upward and forward displacement of the globe. The mass demonstrated a homogeneous signal intensity, similar to that of muscle, on both T1- and T2-weighted images with few small foci of low-T1, high-T2 signals implying cystic changes. Following gadolinium injection, only mild homogeneous enhancement was noted on the T1-weighted images.

The tumor was approached through facial degloving. It appeared as homogeneous pinkish gray granular tissue and had a firm consistency. The lesion was found to originate from the right alveolar ridge and fill the whole maxillary sinus. The lateral and posterior walls of the sinus were destroyed by the tumor, while the orbital floor and the infraorbital nerve canal were not involved and thus were preserved. The tumor was removed piecemeal, the total weight being 90 g. The medial maxillary wall with the inferior turbinate was resected. The ethmoids contained only retained secretions. Due to uncertainty regarding its biologic behavior, it was decided not to sacrifice the alveolar ridge and palate at this stage. The postoperative course was uneventful.

CLASSIFICATION OF FIBRO-OSSEOUS JAW LESIONS

- Fibrous dysplasia
    - Monostotic
    - Polyostotic
- Dysplastic lesions arising in tooth-bearing area (presumably of periodontal ligament origin)
    - Periapical cemento-osseous dysplasia
    - Focal cemento-osseous dysplasia
    - Florid cemento-osseous dysplasia
- Fibro-osseous neoplasms
    - Cemento-ossifying fibroma*
        - Cementifying fibroma
        - Ossifying fibroma
    - Juvenile (cemento-) ossifying fibroma

Modified from Waldron.[1]

*1972 World Health Organization classification separated cementifying fibroma from ossifying fibroma. 1992 World Health Organization classification considers them as histologic variants of same lesion and groups both under single designation as cemento-ossifying fibroma.

Histologic examination showed cementicles and trabeculae of woven bone rimmed by osteoblasts embedded in moderately cellular fibrous stroma. Sharply circumscribed, spherical calcified deposits were scattered throughout the lesion. The pathologic diagnosis was a tumor consistent with cemento-ossifying fibroma.

Four months after surgery a follow-up CT scan revealed slowly growing residual tumor originating from the alveolar ridge remnant. The patient is currently being observed and further surgery is suspended at this point.

## DISCUSSION

Benign fibro-osseous lesions of the jaws present problems in classification, diagnosis, and management. Since the histologic features are similar in a variety of lesions with diverse clinical characteristics and prognosis, precise diagnosis requires clinical and radiologic correlation. A classification scheme is presented in the Table.[1]

Juvenile ossifying fibroma is a relatively rare lesion that can be distinguished by its clinical, radiologic, and histologic characteristics. Most cases involve the maxilla, paranasal sinuses, and orbital and fronto-ethmoidal bones, but mandibular lesions have also been reported. The tumor occurs mostly in children and adolescents, 79% of the patients being 15 years or younger.[2] These lesions are often termed "active" or "aggressive" ossifying fibroma due to their tendency for progressive and sometimes rapid growth and tendency to recur following incomplete excision. When the orbit is involved, either directly or by a sinus lesion, the patient may develop proptosis, exophthalmos, and bulbar displacement.

Radiographically, juvenile ossifying fibroma is monostotic, demarcated from its surroundings with well-defined margins, is round or ovoid, and produces expansion of the involved bone.[2-4] This demarcation from the surrounding bone is an important feature in distinguishing it from fibrous dysplasia. The lesion tends to show areas of radiolucency and fine specks of radiopacity. This is well demonstrated in the current case. In comparison, fibrous dysplasia has a classic ground-glass appearance on radiographs, it expands the bone throughout its length rather than in a localized fashion, its borders are poorly defined, and it is often polyostotic. Depending on the extent of cystic changes and calcifications in the tumor, it will show various degrees of radiolucency and radiopacity, respectively.[1,4] Isolated reports describing the anatomy of the lesion on MRI have appeared in the literature.[4,5] Recently, Kuta et al[4] described the MRI appearance of (central) cemento-ossifying fibroma. In their report, the tumor appeared isointense to muscle on T1-weighted images and hypointense on T2-weighted images. The postgadolinium appearance was not reported. Our case appears isointense to muscle on both T1- and T2-weighted MRI. The isointensity of the tumor to muscle on T2 presumably represents a higher free-water content of the current tumor, and may even represent an innate difference between adult cemento-ossifying fibroma and juvenile ossifying fibroma.

Histologically, juvenile ossifying fibroma shows cellular stroma that produces little collagen. The characteristic mineral component consists of small spherical ossicles that are surrounded by osteoid rims. Some authors stress the osteoid component in establishing the diagnosis.[2,6-9] Others stress the correlation between pathologic and clinical-radiologic features as the basis for diagnosing these lesions.[1,10,11] Slootweg et al[7] differentiate between a variant of juvenile ossifying fibroma with stroma containing psammomalike ossicles and a variant consistent with the World Health Organization definition, containing strands of cellular osteoid. In other cases, maturation of the lesion results in formation of more typical trabeculae of woven bone. Tumors with this histologic appearance have been designated as trabecular desmo-osteoblastoma by Makek.[8] Our case conforms to this histologic variant.

The clinical management of juvenile ossifying fibroma is controversial. Although some authors claim that surgical excision must be complete,[5] others feel that conservative local excision or thorough curettage with preservation of function is the preferable treatment.[1] Spontaneous sarcomatous transformation has not been known for this tumor, so that radical surgery with sacrifice of function does not seem to be indicated. Radiotherapy is not effective, and indeed, it is contraindicated, as it may induce sarcomatous transformation. Reported recurrence rates range from 30% to 58%, in contrast to the negligible recurrence rates associated with the more common types of ossifying fibroma.[1] Recurrence may also be managed by local excision.

In conclusion, we report a case of juvenile cemento-ossifying fibroma of the maxilla. As the histologic features are similar among the benign fibro-osseous lesions, the exact diagnosis was established by the clinical presentation and especially the radiographic appearance.

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## FIFTH INTERNATIONAL COCHLEAR IMPLANT CONFERENCE

The Fifth International Cochlear Implant Conference will be held May 1-3, 1997, in New York, New York. For information, contact New York University Medical Center, Post-Graduate Medical School, 550 First Avenue, New York, NY 10016; telephone (212) 263-5295; fax (212) 263-5293.

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## COPENHAGEN OTITIS MEDIA CONFERENCE

The Copenhagen Otitis Media Conference — Third Extraordinary International Symposium on Recent Advances in Otitis Media will be held in Copenhagen, Denmark, June 1-5, 1997. For more information, contact Copenhagen Otitis Media Conference, Secretariat, ENT Department, Gentofte University Hospital, DK-2900 Hellerup, Denmark; telephone +45 3977 3833; fax +45 3977 7634.

*Ann Otol Rhinol Laryngol* 106:1997

# CLINICOPATHOLOGICAL CONSULTATION

# EAR CHOLESTEATOMA VERSUS CHOLESTEROL GRANULOMA

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**Cholesteatoma and cholesterol granuloma of the ear are two different pathologic conditions that have often been confused in the past, and even in the present, in the literature. The features differentiating the two lesions are presented.**

## INTRODUCTION

Cholesteatoma and cholesterol granuloma continue to pose diagnostic and therapeutic challenges to both pathologists and otologists. The differential diagnosis between these lesions is very important, not only for academic reasons, but also in terms of clinical implications and treatment modalities. In this article we discuss the differential diagnosis between cholesteatoma and cholesterol granuloma in the ear. This distinction has been the subject of much argument and discussion, and often the two lesions have been confused in the literature, particularly in the past. These conditions can occur simultaneously, and this occurrence has led to some speculation and confusion concerning the role of cholesterol in the formation of cholesteatoma.[1]

## CHOLESTEATOMA

*Definition.* Cholesteatoma represents an epidermoid cyst characterized by independent and progressive growth; it can sometimes lie dormant, but very often causes the destruction of adjacent tissue — of bone in particular — and has the tendency to recur despite extensive radical surgery. Therefore, it is not a malignant lesion, but it is nonetheless insidious and potentially dangerous.[2]

*Nomenclature.* The term cholesteatoma is clearly a misnomer for the following reasons: 1) it is not a neoplasm; therefore, the suffix "-oma" is not appropriate; 2) it does not contain cholesterol crystals; therefore, "chole-" is not indicated; and 3) it does not contain fat; therefore, "-stea-" is inappropriate.

The term cholesteatoma was first used by the famous German anatomist Johannes Müller,[3] in 1838, and he meant to describe a true neoplasm; but it was the French pathologist Cruveilhier[4] who, in 1829, described the pathologic features of this lesion. He used the term "pearl tumor," referring to the appearance of the mass. This term was also used by Pinson, an artist in the Paris School of Medicine, in 1807.[5] Almost two centuries earlier, the anatomist Guichard Joseph Du Verney[6] used the term "steatome" to indicate the presence of cholesteatomatous substances in and around the base of the skull.

Cushing[7] used the term "epidermal cholesteatoma," whereas Critchley and Ferguson,[8] who found the growth in the brain and spinal cord, proposed "epidermoid" because it was more suitable to convey its epithelial character.

Various terms have been used to designate this lesion, such as margaritoma, pearly squamous cell carcinoma, cholesteatosis, squamous epitheliosis, epidermosis, epidermoid cholesteatoma, keratoma, keratosis, epidermoid keratinous cyst, keratinous cyst, epidermoid cyst, etc. These definitions reflect the controversy surrounding the origin and the nature of the lesion.

Despite the questions about the correctness of the term cholesteatoma, it is nevertheless retained, for it is deeply embedded in the literature and the language.

*Pathogenesis.* The pathogenesis of cholesteatoma

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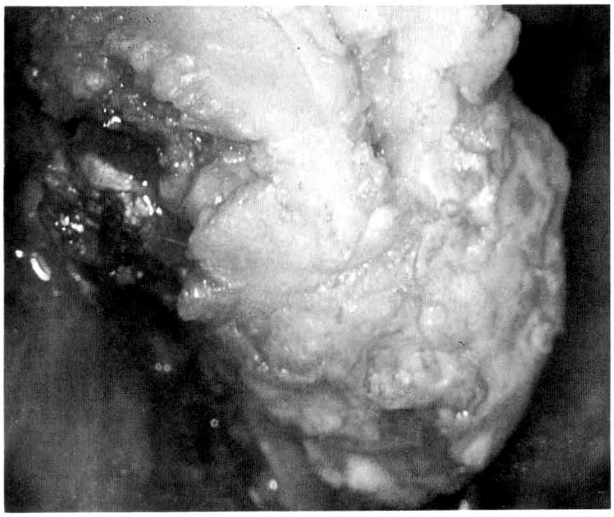

Fig 1. Giant cholesteatoma.

is still debated, and the following theories have been advanced: 1) the congenital theory, 2) the immigration theory (direct extension through a perforation, proliferation of basal cells of canal skin into the submucosa of the tympanum, or attic retraction), 3) the metaplastic theory, and 4) the implantation theory. Several comprehensive discussions of the pathogenesis of cholesteatoma can be found elsewhere.[9-11]

*Pathology*. Macroscopically, the lesion is cystic and appears as a roundish or oval friable mass that is whitish in color, spongy in consistency, and of a variable size and configuration. It may sometimes reach the size of several centimeters or more in greatest dimension (Fig 1), whereas at other times it is so small that its clinical detection is impossible.

It appears histologically as a benign keratinizing squamous cell cyst (Figs 2 and 3). The lesion is basically made up of three components, ie, the cystic content, the matrix (often called capsule or cholesteatoma epithelium), and the perimatrix (called also cholesteatoma stroma). The cystic content is composed of fully differentiated, anucleate, laminated masses of keratin. The matrix of cholesteatoma consists of keratinizing squamous epithelium lining a cystlike structure. The stratified squamous epithelium is characterized by the presence of intercellular bridges, and a regular disposition of the various layers of cells may be found. In particular, the matrix of the cholesteatoma comprises the following:

1. The basal layer or stratum germinativum, made up of columnar epithelium composed of small cuboidal cells displaying an enlarged nucleus with a hyperchromatic or basophilic appearance.
2. The malpighian or spinous layer, composed of larger cells, still relatively cylindrical in shape, which become polyhedral in the more superficial layers.
3. The granular layer, in which cells become progressively flatter and contain hyperchromatic keratohyaline granules in their cytoplasm.
4. The lucidum layer, which is often not detectable.
5. The horny layer, which appears to be clearly hyperkeratotic and desquamating, and in which the lamellae of keratin form the cystic content.

Transition from squamous epithelium, constituting the matrix of the cholesteatoma, to ciliated columnar epithelium with goblet cells can be occasionally seen.[12,13] The perimatrix or lamina propria is the peripheral part of the cholesteatoma and consists of granulation tissue or inflamed subepithelial connective tissue containing inflammatory cells composed of lymphocytes, histiocytes, plasma cells, and, less frequently, neutrophils. Acquired cholesteatomas are

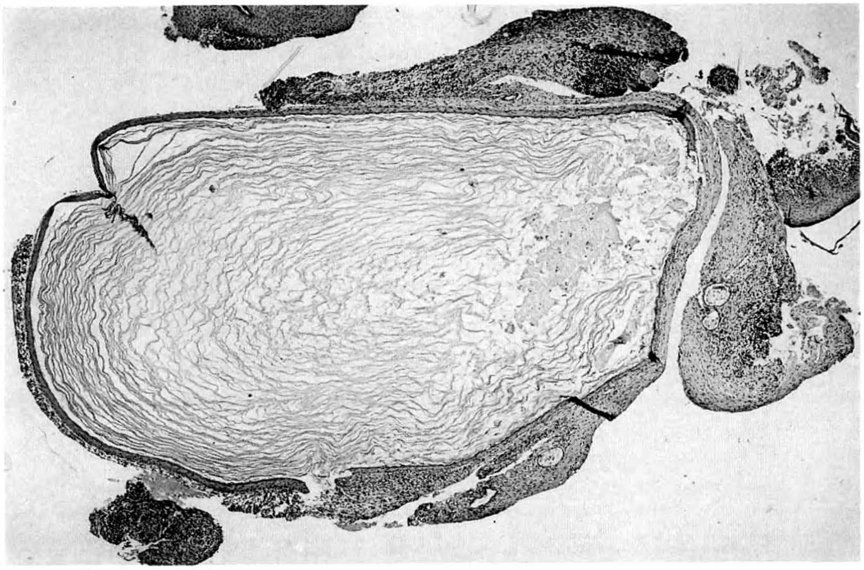

Fig 2. Typical pattern of cholesteatoma. All three components, ie, perimatrix, matrix, and cystic component, are present.

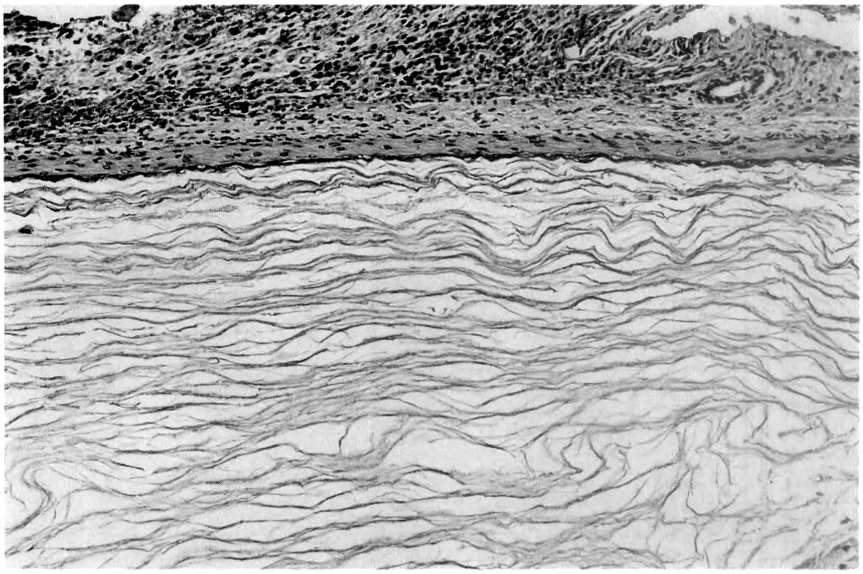

Fig 3. Detail of Fig 2.

often accompanied by glandular metaplasia[14] (Fig 4). If the cholesteatoma sac ruptures, there is spreading of the keratinous substance in the subepithelial layer, and this usually causes a marked granulomatous reaction of the foreign body type. Neither cutaneous adnexa or their remnants nor dermal papillae are ever seen. Osteoclasts are frequently found at the interface between the cholesteatoma matrix and the underlying bone.

All cholesteatomas (acquired or secondary and congenital) exhibit substantially the same morphologic appearance, regardless of their mechanism of formation, but there is a substantially greater thickness of the matrix in the acquired variety than in the congenital variety.[15,16] The matrix of acquired cholesteatoma is usually composed of approximately 15 layers of cells, whereas that of congenital (or primary) cholesteatoma comprises only about 5 layers. In congenital cholesteatoma, there is usually a paucity of acute or chronic inflammation, and glandular structures in the perimatrix are usually absent. In acquired cholesteatomas, there is an absolute increase in the number of dendritic cells within the squamous epithelial component, as compared to the number of dendritic cells that are present in the squamous epithelium of noninflamed congenital cholesteatomas. This was ascertained by utilizing S-100 protein to identify the dendritic cells. Therefore, in this setting the S-100 protein staining of dendritic cells may be useful in the diagnosis of cholesteatomas.[17] The diagnosis of cholesteatoma is made by the presence of matrix, perimatrix, and the cystic component. The presence of keratin debris alone is not diagnostic of a cholesteatoma,[18] even if the presence in the aural polyps of a combination of a foreign

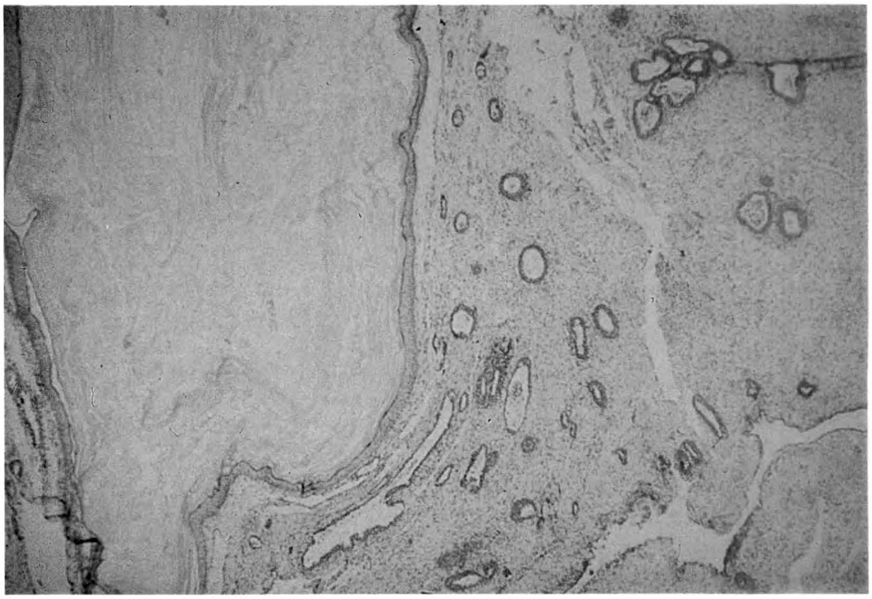

Fig 4. Acquired cholesteatoma in association with glandular metaplasia of middle ear epithelium.

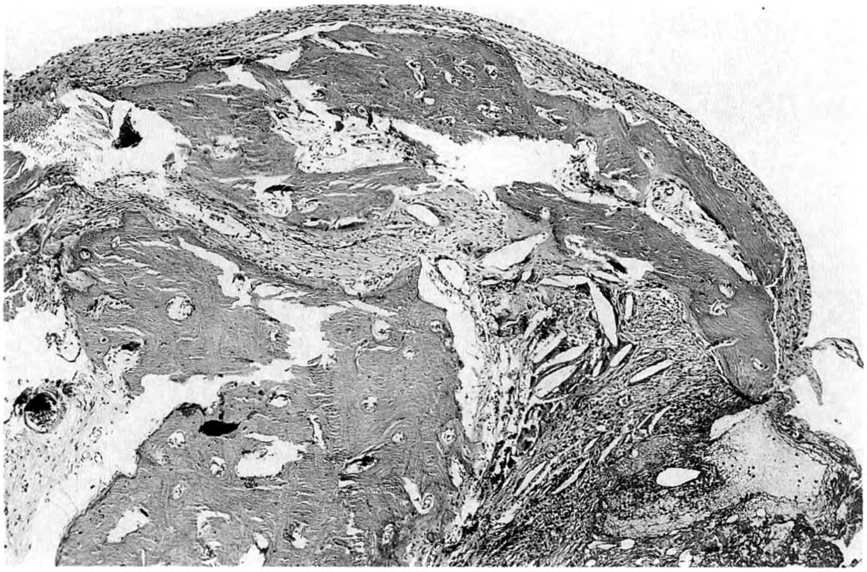

Fig 5. Section of bone fragment with cholesterol granuloma.

body reaction and keratin arranged as masses or flakes is highly suggestive of an underlying cholesteatoma.[19]

Cytokeratins, vimentin, cytokines, and c-myc oncogene expression have been found in acquired middle ear cholesteatomatous lesions.[20-24]

The ultrastructural features are similar to those of normal epidermis. In particular, both Langerhans cells and Merkel cells have been found. The former are located within the prickle-cell layer, between keratinocytes, and the latter within the germinative layer.

Scanning electron microscopy has revealed the presence of corneocytes in the shape of a hexagonal disc, arranged in regular columns, with each column surrounded by six others.[25]

Complete surgical excision of all components of the lesion is the treatment of choice.

## CHOLESTEROL GRANULOMA

*Definition*. Cholesterol granuloma is a granulomatous lesion containing cholesterol crystals.

*Pathogenesis*. The necessary factors for the development of cholesterol granuloma are hemorrhage, interference with clearance or drainage, and obstruction of air exchange or ventilation.[26] Experimental cholesterol granulomas have been observed in the bullae of guinea pigs following the injection of sterile cholesterol suspensions.[1]

*Pathology*. Macroscopically, the lesion shows a round, ovoid, or more irregular appearance with a brownish or yellow color and a glistening aspect.

Microscopically, the lesion is composed of a large number of cholesterol crystals surrounded and engulfed by multinucleated foreign body giant cells and embedded in fibrous granulation tissue (Figs 5 and 6).

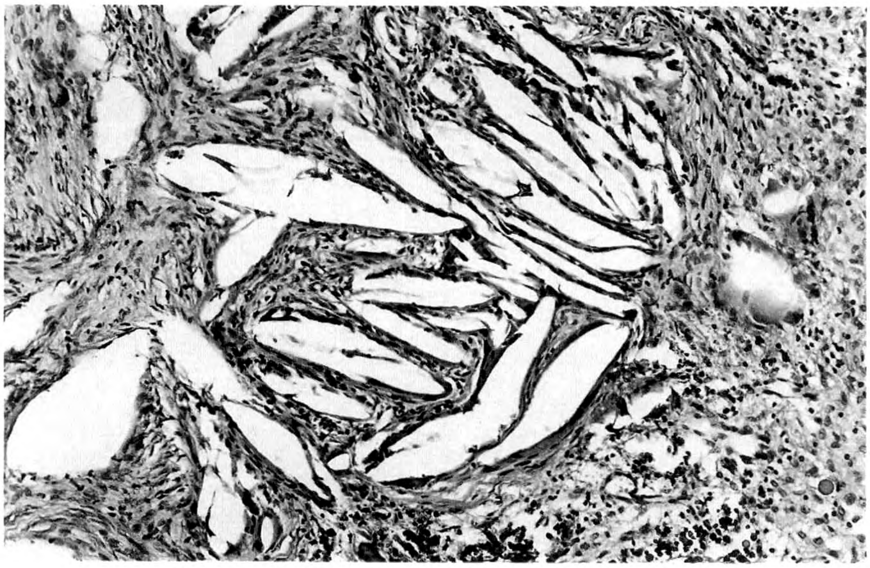

Fig 6. Another cholesterol granuloma.

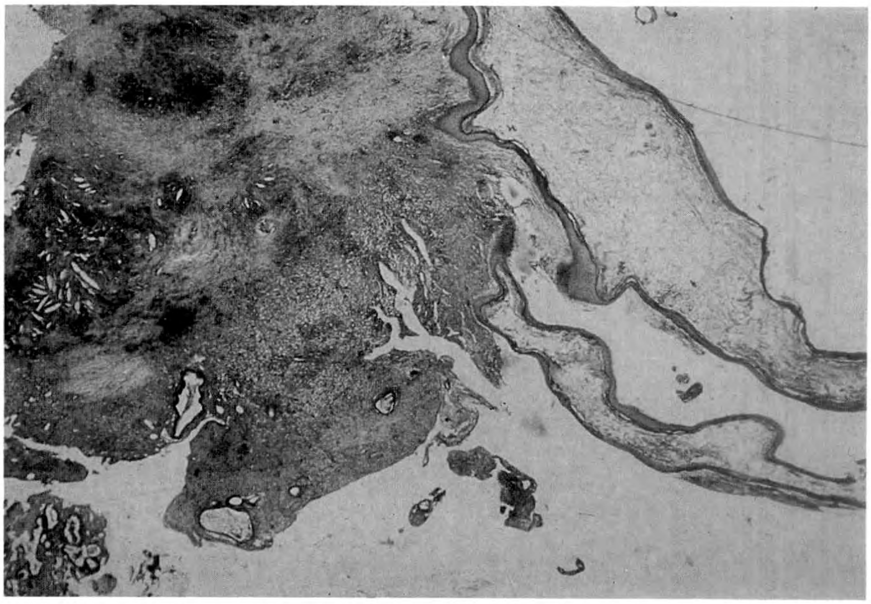

Fig 7. Cholesteatoma and cholesterol granuloma are present in same specimen. This combination is not rare.

The cholesterol is dissolved in the course of processing the tissues for histologic examination. Additional findings include the presence of areas of old and recent hemorrhage with numerous macrophages including hemosiderin.

In the setting of middle ear disease, cholesterol granulomas may be seen in association with cholesteatoma (Fig 7), chronic otitis media, middle ear adenomatous tumors, and endolymphatic sac (Heffner's) tumors.[27]

In fact, cholesterol granulomas are not limited to the middle ear region, but occur throughout the body, including a frequent association with atheromatous thickening of the intima of arteries and in other sites where hemorrhage and/or necrosis have occurred, such as maxillary antrum, thyroid, and kidney.

## DISCUSSION

Cholesteatoma has been mistaken by clinicians and pathologists for cholesterol granuloma.[28] The histopathology of cholesteatoma differs fundamentally from that of cholesterol granuloma, in that the

FEATURES DIFFERENTIATING CHOLESTEATOMA FROM CHOLESTEROL GRANULOMA

| *Cholesteatoma* | *Cholesterol Granuloma* |
|---|---|
| Incidence: frequent | Uncommon |
| Preferred location: middle ear | Petrous apex |
| Composed of perimatrix (fibrous tissue) and matrix (stratified squamous epithelium), and always containing horny lamellae in cystic lumen | Composed of fibrous tissue in which cholesterol crystals may be seen, surrounded by numerous inflammatory cells and by several giant cells of foreign body type |
| Morphologically, epidermoid cyst | Morphologically, reactive foreign body granuloma |
| Basically "epithelial" lesion | "Stromal" lesion |
| May not be connected with chronic inflammatory process | Almost always represents focal response to chronic middle ear infection |
| Computed tomography | |
| Margins: sharp, smooth | Sharp, smooth |
| Density: isodense to cerebrospinal fluid | Isodense to brain |
| Enhancement: no | No |
| Magnetic resonance imaging | |
| T1-weighted signal intensity: low | High |
| T2-weighted signal intensity: high | High |
| Areas of signal void: absent | Present |
| Association with erosion or displacement of ossicles | May be associated with adjacent erosion of ossicles and bone |
| Association with other diseases: tympanosclerosis, atelectasis of ear | Middle ear adenomatous tumors, endolymphatic sac (Heffner's) tumors |
| Treatment: complete surgical excision | Drainage and aeration or radical excision |

Modified from Ferlito.[2]

former is an epidermoid cyst while the latter represents a reactive process in which macrophages are forming giant cells in reaction to a foreign material (ie, lipid released from the cell membranes of red blood cells).[29]

The differential diagnosis between cholesteatoma and cholesterol granuloma is based on distinct clinicopathological features (see Table[2]).

Cholesteatoma is a common epithelial lesion localized predominantly in the middle ear cleft with an epidermoid cystic structure. It is composed of a basal layer characterized by keratinizing squamous epithelium ("matrix" or capsule), of a thick subepithelial layer that consists of inflamed fibrous tissue ("perimatrix" or lamina propria), and of a cystic lumen containing desquamated keratin accumulated in an onionlike fashion. It may be associated with a chronic inflammatory process.

Cholesterol granuloma is an uncommon stromal lesion that is most frequently found in the petrous apex. It is a foreign body granuloma almost always representing a focal reaction to chronic middle ear infection. It is composed of numerous clefts of cholesterol crystals surrounded by several foreign body giant cells embedded in fibrous tissue with inflammatory cells and areas of old and recent hemorrhage.

The computed tomography scan appearance of cholesteatoma may be similar to that of cholesterol granuloma. Both the lesions may appear with sharp and smooth margins, with an identical density, and with a lack of enhancement by intravenous contrast medium. The opacification of cholesterol granuloma has the same density as brain and does not enhance with contrast.

Magnetic resonance imaging may distinguish between cholesteatoma and cholesterol granuloma. The former shows isodensity or hypodensity on T1-weighted images and high signal intensity on T2-weighted images, while the latter is characterized by high signal intensity on both T1- and T2-weighted images because of the paramagnetic effects of protein content and cholesterol,[30-32] and by the presence of areas of void.

Cholesteatomas are associated with destruction of bony structures, while cholesterol granulomas may occur in association with adjacent erosion of ossicles and bones. The destructive properties of cholesteatoma are caused by production of collagenase by both the squamous epithelial and fibrous tissue components.[18]

Cholesteatoma may be associated with tympanosclerosis and atelectasis of the middle ear, while cholesterol granulomas may occur in a wide setting of middle ear lesions including otitis media, middle ear adenomatous tumors, and endolymphatic sac (Heffner's) tumors.

Complete surgical excision of all components of cholesteatoma is necessary. Attempts to remove cholesteatoma matrix in large labyrinthine fistulas place the hearing at high risk. In operations on the only hearing ear, it is relatively safe to leave cholesteatoma matrix over the fistula in an open cavity procedure. Despite the presence of the matrix, healing occurs once the pressure and infection have been eliminated. Petrous bone cholesteatomas, involving the petrous portion of the temporal bone, may be a difficult challenge, owing to the potential involvement of the facial nerve, otic capsule, jugular bulb, carotid artery, and middle and posterior fossa dura.[33] In small, supralabyrinthine cholesteatomas, a middle cranial fossa approach combined with a transmastoid approach and cavity marsupialization is the treatment of choice. In infralabyrinthine cholesteatomas with preserved inner ear function, a subtotal petrosectomy[34] or an infratemporal fossa type B approach[35] is indicated. In massive petrous bone cholesteatomas with destruction of the labyrinth, a modified transcochlear approach is indicated.[33]

Cholesterol granulomas do not require aggressive management. Drainage and permanent aeration are often useful.[32,36] For the treatment of cholesterol granuloma of the petrous apex, a variety of surgical procedures have been described. The choice of surgical approach depends on the size of the lesion, the symptoms, the status of the patient's hearing, the age of the patient, and the preference of the surgeon.[37] The middle fossa extradural approach[38] may be used, possibly combined with a transmastoid approach. The transmastoid-infralabyrinthine approach is the procedure of choice when hearing preservation is desired and the jugular bulb is not too high[33,39]; the infratemporal fossa type B approach[35] is preferable when cochlear function may be saved; and the modified transcochlear approach[33] is used when hearing cannot be saved. Small, asymptomatic cholesterol granulomas of the petrous apex should not be operated on, but should be followed up on a yearly basis with computed tomography scanning and magnetic resonance imaging.

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# BOOK REVIEWS

## Surgical Pathology of Laryngeal Neoplasms

Alfio Ferlito, editor. Hard cover, illustrated, indexed, 486 pages, 1996. Chapman & Hall Medical, Oxford, England.

It is not very often that a sequel in the motion picture industry maintains commercial appeal and outdoes the previous box office grosses. However, when it does happen, both producers and moviegoers are rewarded with an exceptional performance. Of course, a sequel maintains a continuity with the characters or the story, elaborating on both, presenting new and diverse challenges to impress the audience.

*Surgical Pathology of Laryngeal Neoplasms*, edited by Alfio Ferlito, comes out as a sequel or rerun to *Neoplasms of the Larynx* (Churchill Livingstone), published in 1993. However, the current "sequel" expands on the pathology content, updates the references, includes color photography, and seems to be geared to pathologists more than to otolaryngic surgeons. An award-winning cast of contributors augment the editor in presenting an evenly paced, easy to read, and comprehensive review of laryngeal neoplasms.

The neoplasms of the larynx are arranged in a logical developmental arc, the more frequent tumors occupying more "time on screen." There is internal consistency in style, obviously accomplished under the direction of the editor. Each neoplasm (or in some instances, each chapter, when devoted to a single entity) is introduced, followed by an overview of the terminology used and a definition of the tumor. Vignettes about the incidence, age and gender predilection, site of occurrence, and clinical presentation are catalogued. By far and away the preponderance of the "script" is devoted to the elements of histogenesis, pathology, and special studies (histochemical stains, immunohistochemical reactions, electron microscopy, molecular testing, and other investigations). Elegant descriptions of the histologic findings, as well as special studies utilized to prove histogenesis or differentiation, are interwoven with photographs of remarkable clarity, contrast, and content. A brief discussion of the differential diagnosis nicely draws to a close the surgical pathologist's approach to each neoplasm before the treatment and prognosis is detailed. From a form standpoint, the inventive inclusion in the upper right-hand corner of a "running subtitle" for easy reference was most helpful and could only have been improved by likewise including the chapter number.

Every book has some problems, and while most of the chapters have many more pages of "dialogue" than pictures, the chapter on neuroendocrine lesions must represent the editor's idiosyncratic pride and joy. Indeed, this is one of only two chapters in which bold print is effectively used to highlight specific entities within the text. It was very easy to find each neoplasm, just by scanning each of the brief 15 pages of text (excluding bibliography) for the boldfaced type. The chapter exclusively covers the topic of neuroendocrine tumors (which is superb), and also contains the book's preponderance of color pictures — 35 of a total of 42 — of the one-dimensional histologic or immunohistochemical characteristics of this scarce entity, some of which are not of the high quality as elsewhere.

The photographs are generally well chosen, of exceptional quality, demonstrating the essential diagnostic features of each neoplasm, presented in potent contrast, with good cellular and nuclear detail — although a number of the photographs are exact duplicates of those used in the previous book (mentioned above). Perhaps this proves the old adage: "If it ain't broke, don't fix it." Perhaps if there is to be another "sequel" in this ongoing series of neoplasms of the larynx, a more even distribution of the photographs between each entity, including an equitable distribution of the color photographs, should be encouraged.

Although the references are quite encyclopedic for the seminal and early papers describing each tumor, there are no references more recent than 1994. With the speed of modern production, it seems that at least some of the references from 1995 could have made it into the final editor's cut. The "production" ends with a short but accurate and inclusive index, which is also cross-referenced.

As with any critical review of a film, this should not dissuade you from seeing the movie for yourself. After all, a review is only a single perception of the reality a director, producer, screenwriter, and cinematographer are trying to conjure in the viewer's mind. Even though there may be some structural glitches in this "production," I would wholeheartedly recommend the acquisition of this relevant treatise to the target audience of pathologists and otolaryngologists, but only if they do not already own the first book in the series.

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Washington, DC

(The opinions or assertions expressed above are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Navy, Department of the Army, or Department of Defense.)

## Occupational Hearing Loss

Volume 10, No. 3. Thais C. Morata and Derek E. Dunn. Hard cover, illustrated, indexed, 97 pages, 1995. Hanley and Belfus, Philadelphia, Pa, $36.

This book is one in a series of monographs on occupational medicine in which state-of-the-art reviews on occupational hearing conservation (OHC) are discussed. Indeed, in its extensive bibliography, the dates of publications show recent developments in the subject of occupational hearing loss.

The scope of the text can be divided into three parts. First are the sociological consequences for those with occupational hearing loss, including what animal models reveal about the potential hazards and effects of noise exposure. Second, chapters are devoted to the effects on hearing of interrelated conditions (physical and chemical agents). Finally, there is coverage of the legislative background of OHC programs.

The emphasis of topics in this text is on the effects of industrial solvents on hearing, and certainly this association is a recent one. Animal experiments have proven the ototoxicity of toluene, xylene, styrene, trichloroethylene, and other solvents in rats. The documentation of the effects of solvents on the auditory system of humans is based on case reports and subjective findings, and minimal findings on pure tone thresholds. The exploration of industrial solvents exposure could pose yet another challenge in sorting out the causes of hearing loss, just as in the relationships of noise and presbycusis or noise-induced hearing loss and acoustic trauma. I am not sure that the investigators have yet made a case, in humans, for susceptibility of hearing to solvents.

The editors have presented a well-written, concise text that primarily should be of interest to students and practitioners in the hearing sciences who are involved with OHC.

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Birmingham, Alabama

VOLUME 106 (2) — FEBRUARY 1997

— *Founded in 1892* —

# ANNALS

# OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

*Official Organ of the*
AMERICAN LARYNGOLOGICAL ASSOCIATION
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SILENT LARYNGOPHARYNGEAL SENSORY DEFICITS AFTER STROKE

SURGICAL MORBIDITY OF NECK DISSECTION AFTER CHEMORADIOTHERAPY IN ADVANCED HEAD AND NECK CANCER

CHRONIC MAXILLARY ATELECTASIS

RUPTURED PEXIS AFTER SUPRACRICOID PARTIAL LARYNGECTOMY

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**AUGMENTIN® amoxicillin/clavulanate potassium**
**BRIEF SUMMARY. FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.**

**INDICATIONS AND USAGE:** *Augmentin* is indicated in the treatment of infections caused by susceptible strains of the designated organisms in the conditions listed below:

*Lower Respiratory Tract Infections* caused by β-lactamase-producing strains of *Haemophilus influenzae* and *Moraxella (Branhamella) catarrhalis*. *Otitis Media* caused by β-lactamase-producing strains of *Haemophilus influenzae* and *Moraxella (Branhamella) catarrhalis*. *Sinusitis* caused by β-lactamase-producing strains of *Haemophilus influenzae* and *Moraxella (Branhamella) catarrhalis*. *Skin and Skin Structure Infections* caused by β-lactamase-producing strains of *Staphylococcus aureus, Escherichia coli*, and *Klebsiella* spp. *Urinary Tract Infections* caused by β-lactamase-producing strains of *E. coli, Klebsiella* spp. and *Enterobacter* spp. While *Augmentin* is indicated only for the conditions listed above, infections caused by ampicillin-susceptible organisms are also amenable to *Augmentin* treatment due to its amoxicillin content. Therefore, mixed infections caused by ampicillin-susceptible organisms and β-lactamase-producing organisms susceptible to *Augmentin* should not require the addition of another antibiotic. Because amoxicillin has greater *in vitro* activity against *Streptococcus pneumoniae* than does ampicillin or penicillin, the majority of *S. pneumoniae* strains with intermediate susceptibility to ampicillin or penicillin are fully susceptible to amoxicillin and *Augmentin*. (See Microbiology subsection.)

Bacteriological studies to determine the causative organisms and their susceptibility to *Augmentin* should be performed together with any indicated surgical procedures. Therapy may be instituted prior to obtaining the results from bacteriological and susceptibility studies to determine the causative organisms and their susceptibility to *Augmentin* when there is reason to believe the infection may involve any of the β-lactamase-producing organisms listed above. Once results are known, adjust therapy, if appropriate.

**CONTRAINDICATIONS:** Patients with a history of allergic reactions to any penicillin; or patients with a history of *Augmentin*-associated cholestatic jaundice/hepatic dysfunction.

**WARNINGS:** SERIOUS AND OCCASIONALLY FATAL HYPERSENSITIVITY (ANAPHYLACTIC) REACTIONS HAVE BEEN REPORTED IN PATIENTS ON PENICILLIN THERAPY. THESE REACTIONS ARE MORE LIKELY TO OCCUR IN INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY AND/OR A HISTORY OF SENSITIVITY TO MULTIPLE ALLERGENS. THERE HAVE BEEN REPORTS OF INDIVIDUALS WITH A HISTORY OF PENICILLIN HYPERSENSITIVITY WHO HAVE EXPERIENCED SEVERE REACTIONS WHEN TREATED WITH CEPHALOSPORINS. BEFORE INITIATING THERAPY WITH *AUGMENTIN*, CAREFUL INQUIRY SHOULD BE MADE CONCERNING PREVIOUS HYPERSENSITIVITY REACTIONS TO PENICILLINS, CEPHALOSPORINS OR OTHER ALLERGENS. IF AN ALLERGIC REACTION OCCURS, *AUGMENTIN* SHOULD BE DISCONTINUED AND THE APPROPRIATE THERAPY INSTITUTED. **SERIOUS ANAPHYLACTIC REACTIONS REQUIRE IMMEDIATE EMERGENCY TREATMENT WITH EPINEPHRINE. OXYGEN, INTRAVENOUS STEROIDS AND AIRWAY MANAGEMENT, INCLUDING INTUBATION, SHOULD ALSO BE ADMINISTERED AS INDICATED. Pseudomembranous colitis has been reported with nearly all antibacterial agents, including *Augmentin*, and has ranged in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.** Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic associated colitis." After the diagnosis of pseudomembranous colitis has been established, appropriate therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation and treatment with an antibacterial drug clinically effective against *Clostridium difficile* colitis. Use *Augmentin* cautiously in patients with evidence of hepatic dysfunction. Hepatic toxicity associated with *Augmentin* use is usually reversible. On rare occasions, deaths have been reported (less than 1 death reported per estimated 4 million prescriptions worldwide). These have generally been cases associated with serious underlying diseases or concomitant medications. (See CONTRAINDICATIONS and ADVERSE REACTIONS.)

**PRECAUTIONS: General:** While *Augmentin* possesses the characteristic low toxicity of the penicillin group of antibiotics, periodic assessment of organ system functions, including renal, hepatic and hematopoietic function, is advisable during prolonged therapy.

A high percentage of patients with mononucleosis who receive ampicillin develop an erythematous skin rash. Thus, ampicillin class antibiotics should not be administered to patients with mononucleosis. The possibility of superinfections with mycotic or bacterial pathogens should be kept in mind during therapy. If superinfections occur (usually involving *Pseudomonas* or *Candida*), the drug should be discontinued and/or appropriate therapy instituted.

**Drug Interactions:** Probenecid decreases the renal tubular secretion of amoxicillin. Concurrent use with *Augmentin* may result in increased and prolonged blood levels of amoxicillin. Co-administration of probenecid cannot be recommended. The concurrent administration of allopurinol and ampicillin increases substantially the incidence of rashes in patients receiving both drugs as compared to patients receiving ampicillin alone. It is not known whether this potentiation of ampicillin rashes is due to allopurinol or the hyperuricemia present in these patients. There are no data with *Augmentin* and allopurinol administered concurrently.

**Drug/Laboratory Test Interactions:** Oral administration of *Augmentin* will result in high urine concentrations of amoxicillin. High urine concentrations of ampicillin may result in false-positive reactions when testing for the presence of glucose in urine using Clinitest®, Benedict's Solution or Fehling's Solution. Since this effect may also occur with amoxicillin and therefore *Augmentin*, it is recommended that glucose tests based on enzymatic glucose oxidase reactions (such as Clinistix® or Tes-Tape®) be used.

Following administration of ampicillin to pregnant women a transient decrease in plasma concentration of total conjugated estriol, estriol-glucuronide, conjugated estrone and estradiol has been noted. This effect may also occur with amoxicillin and therefore *Augmentin*.

**Carcinogenesis, Mutagenesis, Impairment of Fertility:** Long-term studies in animals have not been performed to evaluate carcinogenic potential. **Mutagenesis:** The mutagenic potential of *Augmentin* was investigated *in vitro* with an Ames test, a human lymphocyte cytogenetic assay, a yeast test and a mouse lymphoma forward mutation assay, and *in vivo* with mouse micronucleus tests and a dominant lethal test. All were negative apart from the *in vitro* mouse lymphoma assay where weak activity was found at very high, cytotoxic concentrations.

**Impairment of Fertility:** *Augmentin* at oral doses of up to 1200 mg/kg/day (5.7 times the maximum human dose, 1480 $mg/m^2$/day, based on body surface area) was found to have no effect on fertility and reproductive performance in rats dosed with a 2:1 ratio formulation of amoxicillin:clavulanate.

**Teratogenic effects: Pregnancy (Category B):** Reproduction studies performed in pregnant rats and mice given *Augmentin* at oral dosages up to 1200 mg/kg/day, equivalent to 7200 and 4080 $mg/m^2$/day, respectively (4.9 and 2.8 times the maximum human oral dose based on body surface area), revealed no evidence of harm to the fetus due to *Augmentin*. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, use this drug during pregnancy only if clearly needed.

**Labor and Delivery:** Oral ampicillin class antibiotics are generally poorly absorbed during labor. Studies in guinea pigs have shown that intravenous administration of ampicillin decreased the uterine tone, frequency of contractions, height of contractions and duration of contractions. However, it is not known whether the use of *Augmentin* in humans during labor or delivery has immediate or delayed adverse effects on the fetus, prolongs the duration of labor, or increases the likelihood that forceps delivery or other obstetrical intervention or resuscitation of the newborn will be necessary.

**Nursing Mothers:** Ampicillin class antibiotics are excreted in the milk; therefore, caution should be exercised when *Augmentin* is administered to a nursing woman.

**ADVERSE REACTIONS:** *Augmentin* is generally well tolerated. The majority of side effects observed in clinical trials were mild and transient; <3% of patients discontinued therapy because of drug-related side effects. The most frequently reported adverse effects were diarrhea/loose stools (9%), nausea (3%), skin rashes and urticaria (3%), vomiting (1%) and vaginitis (1%). The overall incidence of side effects, and in particular diarrhea, increased with the higher recommended dose. Other less frequently reported reactions include: abdominal discomfort, flatulence and headache.

The following adverse reactions have been reported for ampicillin class antibiotics:

Diarrhea, nausea, vomiting, indigestion, gastritis, stomatitis, glossitis, black "hairy" tongue, enterocolitis, mucocutaneous candidiasis and pseudomembranous colitis. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment. (See WARNINGS.) Skin rashes, pruritus, urticaria, angioedema, serum sickness-like reactions (urticaria or skin rash accompanied by arthritis, arthralgia, myalgia and frequently fever), erythema multiforme (rarely Stevens-Johnson Syndrome) and an occasional case of exfoliative dermatitis (including toxic epidermal necrolysis). These reactions may be controlled with antihistamines and, if necessary, systemic corticosteroids. Whenever such reactions occur, the drug should be discontinued, unless the opinion of the physician dictates otherwise. Serious and occasional fatal hypersensitivity (anaphylactic) reactions can occur with oral penicillin. (See WARNINGS.) A moderate rise in AST (SGOT) and/or ALT (SGPT) has been noted in patients treated with ampicillin class antibiotics but the significance of these findings is unknown. Hepatic dysfunction, including increases in serum transaminases (AST and/or ALT), serum bilirubin and/or alkaline phosphatase, has been infrequently reported with *Augmentin*. The histologic findings on liver biopsy have consisted of predominantly cholestatic, hepatocellular, or mixed cholestatic-hepatocellular changes. The onset of signs/symptoms of hepatic dysfunction may occur during or several weeks after therapy has been discontinued. The hepatic dysfunction, which may be severe, is usually reversible. On rare occasions, deaths have been reported (less than 1 death reported per estimated 4 million prescriptions worldwide). These have generally been cases associated with serious underlying diseases or concomitant medications. Interstitial nephritis and hematuria have been reported rarely. Anemia, thrombocytopenia, thrombocytopenic purpura, eosinophilia, leukopenia and agranulocytosis have been reported during therapy with penicillins. These reactions are usually reversible on discontinuation of therapy and are believed to be hypersensitivity phenomena. A slight thrombocytosis was noted in less than 1% of the patients treated with *Augmentin*. Reversible hyperactivity, agitation, anxiety, insomnia, confusion, behavioral changes, and/or dizziness have been reported rarely.

**DOSAGE AND ADMINISTRATION**

**Since both the *Augmentin* 250 mg and 500 mg tablets contain the same amount of clavulanic acid (125 mg, as the potassium salt), 2 *Augmentin* 250 mg tablets are not equivalent to 1 *Augmentin* 500 mg tablet. Therefore, 2 *Augmentin* 250 mg tablets should not be substituted for 1 *Augmentin* 500 mg tablet.**

**Dosage:**

**Adults:** The usual adult dose is 1 *Augmentin* 500 mg tablet every 12 hours or 1 *Augmentin* 250 mg tablet every 8 hours. For more severe infections and infections of the respiratory tract, the dose should be 1 *Augmentin* 875 mg tablet every 12 hours or 1 *Augmentin* 500 mg tablet every 8 hours.

Patients with impaired renal function do not generally require a reduction in dose unless the impairment is severe. Severely impaired patients with a glomerular filtration rate of <30 mL/minute should not receive the 875 mg tablet. Patients with a glomerular filtration rate of 10 to 30 mL/minute should receive 500 mg or 250 mg every 12 hours, depending on the severity of the infection. Patients with a less than 10 mL/minute glomerular filtration rate should receive 500 mg or 250 mg every 24 hours, depending on severity of the infection.

Hemodialysis patients should receive 500 mg or 250 mg every 24 hours, depending on severity of the infection. They should receive an additional dose both during and at the end of dialysis.

Hepatically impaired patients should be dosed with caution and hepatic function monitored at regular intervals. (See WARNINGS.)

**Pediatric Patients:** Pediatric patients weighing 40 kg or more should be dosed according to the adult recommendations.

**Due to the different amoxicillin to clavulanic acid ratios in the *Augmentin* 250 mg tablet (250/125) versus the *Augmentin* 250 mg chewable tablet (250/62.5), the *Augmentin* 250 mg tablet should not be used until the pediatric patient weighs at least 40 kg or more.**

**Administration:** *Augmentin* may be taken without regard to meals; however, absorption of clavulanate potassium is enhanced when *Augmentin* is administered at the start of a meal. To minimize the potential for gastrointestinal intolerance, *Augmentin* should be taken at the start of a meal.

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**Please see brief summary of prescribing information for contraindications, warnings, precautions, adverse reactions, and dosage and administration.**

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**References: 1.** Neu HC, Wilson APR, Grüneberg RN. Amoxycillin/clavulanic acid: a review of its efficacy in over 38,500 patients from 1979 to 1992. *J Chemother* 1993;5:67-93. **2.** *Clinical Efficacy Updates I-IV*, 1996, SmithKline Beecham Pharmaceuticals.

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AG8737

ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 106 FEBRUARY 1997 NUMBER 2

# CONTENTS

## FROM THE AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

**Silent Laryngopharyngeal Sensory Deficits After Stroke**
Jonathan E. Aviv, MD; Ralph L. Sacco, MD, MS; Jeanne Thomson, MS;
Raj Tandon, MD; Beverly Diamond, PhD; John H. Martin, PhD; Lanny G. Close, MD ..... 87

## FROM THE AMERICAN LARYNGOLOGICAL ASSOCIATION

**Cartilaginous Tumors of the Larynx: Clinicopathologic Review of 47 Cases**
Jean E. Lewis, MD; Kerry D. Olsen, MD; Carrie Y. Inwards, MD ..... 94

**Superior Laryngeal Nerve Brain Stem Evoked Response in the Cat**
Young-Ho Kim, MD, PhD; Won Pyo Hong, MD, PhD;
Kwang-Moon Kim, MD, PhD; Hong Yoon Kim, MD ..... 101

## INDEPENDENT CONTRIBUTIONS

**Chronic Maxillary Atelectasis**
Erik S. Kass, MD; Salah Salman, MD; Peter A. D. Rubin, MD;
Alfred L. Weber, MD; William W. Montgomery, MD ..... 109

**Surgical Morbidity of Neck Dissection After Chemoradiotherapy in Advanced Head and Neck Cancer**
James P. Newman, MD; David J. Terris, MD; Harlan A. Pinto, MD;
Willard E. Fee, Jr, MD; Richard L. Goode, MD; Donald R. Goffinet, MD ..... 117

**Usher's Syndrome Type 1C: Clinical Studies and Fine-Mapping the Disease Locus**
Jacquie Marietta; Katherine S. Walters; Robert Burgess, MD;
Li Ni, MD; Kunihiro Fukushima, MD; Kenneth C. Moore, PhD;
J. Fielding Hejtmancik, MD, PhD; Richard J. H. Smith, MD ..... 123

**Vestibular Abnormalities in CHARGE Association**
Toshihisa Murofushi, MD; Robert A. Ouvrier, MD; Geoffrey D. Parker, MB BS;
Robert I. Graham, MB BS; Merl da Silva, MB BS; G. Michael Halmagyi, MD ..... 129

**Rhinolith: An Unusual Case and an Update**
András Ézsiás, MD, FRCS; Adrian W. Sugar, FDSRCS ..... 135

**Summating Potential and Action Potential Gradients on and in the Vicinity of the Round Window in Guinea Pig**
Mohamed Badr-El-Dine, MD; George M. Gerken, PhD; William L. Meyerhoff, MD, PhD ..... 139

**Glucose Utilization of the Rat Vestibular End Organs: A Quantitative 2-Deoxyglucose Study**
Michael J. Olds, MD; Michael J. Lyon, PhD ..... 145

**Morbidity of Combined Therapy for the Treatment of Supraglottic Carcinoma: Supraglottic Laryngectomy and Radiotherapy**
Joseph R. Steiniger, MD; Steven M. Parnes, MD; Glendon M. Gardner, MD ..... 151

## MAJOR SURGICAL COMPLICATIONS

**Ruptured Pexis After Supracricoid Partial Laryngectomy**
Ollivier Laccourreye, MD; Daniel Brasnu, MD;
Laurent Laccourreye, MD; Gregory Weinstein, MD ........ 159

## IMAGING CASE STUDIES OF THE MONTH

**Odontogenic Keratocyst in a Young Child**
Samir S. Shah; John G. Meara, MD, DMD; Hugh D. Curtin, MD;
Kasey K. Li, DDS, MD; Michael J. Cunningham, MD ........ 163

**Sonography in Preauricular Pilomatrixoma of Childhood**
A. Michelle Fink, MRCP, FRCR; Robert G. Berkowitz, FRACS ........ 167

## PATHOLOGY CONSULTATION

**Intracellular Crystalline Deposits in Lymphoplasmacellular Disorders**
Patricia Suarez, DDS; Adel K. El-Naggar, MD, PhD; John G. Batsakis, MD ........ 170

## MISCELLANEOUS

**Acknowledgment of Reviewers** ........ 173

**Information for Subscribers** ........ 1a

**Index to Advertisers** ........ 10a

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# SILENT LARYNGOPHARYNGEAL SENSORY DEFICITS AFTER STROKE

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Dysphagia and aspiration are two devastating sequelae of stroke. Recent work has shown that laryngopharyngeal (LP) sensory deficits are associated with aspiration in stroke patients with dysphagia. The phenomenon of silent LP sensory deficits, where the patient exhibits no subjective or objective evidence of dysphagia, yet has an LP sensory deficit, has not been previously described. The aim of this study was to evaluate the sensory capacity of the laryngopharynx in stroke patients who had no subjective or objective complaints of dysphagia. We determined the sensory threshold in the laryngopharynx using air pulse stimulation of the mucosa of the pyriform sinus and aryepiglottic fold. Eighteen stroke patients (mean age 65.6 ± 11.5 years) and 18 age-matched controls were prospectively evaluated. Normal thresholds were defined as <4.0 mm Hg air pulse pressure (APP). Deficits were defined as either a moderate impairment in sensory discrimination thresholds (4.0 to 6.0 mm Hg APP) or a severe sensory impairment (>6.0 mm Hg APP). Stroke patients were followed up for 1 year to determine the incidence of aspiration pneumonia (AP) as verified by chest radiography. In 11 of the stroke patients studied, either unilateral ($n = 6$) or bilateral ($n = 5$) severe sensory deficits were identified. The elevations in sensory discrimination thresholds were significantly greater than those in age-matched controls (7.1 ± 0.6 mm Hg APP versus 2.5 mm Hg APP; $p < .01$, Wilcoxon score). Among patients with unilateral deficits, sensory thresholds were severely elevated in all cases on the affected side compared with the unaffected side ($p < .01$, Wilcoxon score). Moreover, the sensory thresholds of the unaffected side were not significantly different from those of age-matched controls. Aspiration pneumonia did not occur in the patients with normal LP sensation or in the patients with unilateral severe LP sensory deficits. However, in the 5 patients with bilateral, severe LP sensory deficits, 2 developed AP, both within 3 months of their LP sensory test. The results of this study showed, for the first time, that stroke patients without subjective or objective clinical evidence of dysphagia could have silent LP sensory deficits. These impairments could contribute to the development of AP following stroke. The findings in this study suggest that LP sensory discrimination threshold testing should not be restricted only to patients with clinical dysphagia.

KEY WORDS — aspiration, dysphagia, laryngopharyngeal sensory deficit, stroke, swallowing.

## INTRODUCTION

Dysphagia is a common and devastating complication of stroke, occurring in approximately one third of all stroke patients.[1,2] Dysphagia may lead to aspiration, pneumonia, and death.[3-5] Aspiration pneumonia (AP) after stroke kills approximately 40,000 people each year in the United States.[5-7]

While approximately 20% of stroke patients with dysphagia develop AP, a surprising 10% of stroke patients without dysphagia also develop AP.[6,8-10] While motor dysfunction in stroke patients clearly can lead to development of dysphagia and AP, we have recently demonstrated that laryngopharyngeal (LP) sensory dysfunction also plays a role.[11] To study LP sensory capacity, we have developed a method to stimulate the mucosa innervated by the superior laryngeal nerve (SLN) using air pulse stimuli.[12,13] Stimuli are delivered to this region through a port in a specially designed fiber-optic laryngoscope. Using this testing method, we have shown that stroke patients with dysphagia have elevated sensory thresholds in the laryngopharynx.[11]

It is not known what factors contribute to the development of AP in the 10% of stroke patients without dysphagia. Just as the phenomenon of silent aspiration after stroke has been described,[14] we postulated that silent LP sensory deficits could also exist whereby a patient who has no specific subjective or objective findings of dysphagia or aspiration has LP sensory deficits on LP sensory discrimination testing. We hypothesized that some stroke patients have silent LP sensory deficits and that this was predictive of subsequent AP. To initiate a test of this hypothesis, we carried out a prospective study of LP sensory

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From the Department of Otolaryngology–Head and Neck Surgery (Aviv, Tandon, Close), the Departments of Neurology and Public Health (Epidemiology) in the Sergievsky Center (Sacco), the Department of Speech Pathology (Thomson), the Irving Center for Clinical Research (Diamond), and the Center for Neurobiology and Behavior, Department of Psychiatry (Martin), Columbia-Presbyterian Medical Center; and the College of Physicians and Surgeons, Columbia University; New York, New York. This work is supported by National Institutes of Health grant RR 00645, the Florence Irving Assistant Professorship, and the Columbia-Presbyterian Medical Center Office of Clinical Trials.

Presented at the meeting of the American Broncho-Esophagological Association, Orlando, Florida, May 6-7, 1996.

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discriminative capacity in stroke patients without subjective or objective complaints of dysphagia and followed them for 1 year to determine the incidence of AP.

## MATERIALS AND METHODS

We prospectively studied 18 acute ischemic stroke patients admitted over a 1-month period in March 1995 to the Neurovascular Unit at the Columbia-Presbyterian Medical Center who had no subjective or objective complaints of dysphagia and who were on regular diets. Dysphagia was defined as any subjective or objective patient complaint of difficulty swallowing solid or liquid food, or the presence of coughing or choking while swallowing. Eighteen age-matched controls were prospectively studied and selected from outpatient volunteers who were on regular diets and had no dysphagia and no history of stroke. Laryngopharyngeal sensory testing was performed in all patients within 4 weeks of stroke onset. All patients studied were on regular diets at the time of LP sensory testing and continued on regular diets after testing.

Two methods were used to determine LP sensory discrimination thresholds in both the test subjects (patients) and the control subjects. Each method used air pulse stimulation of pyriform mucosa and aryepiglottic (AE) folds (the region innervated by the SLN). One method, psychophysical testing, has been described previously.[11-13] The second method was to elicit the laryngeal closure reflex as a measure of determining LP sensory thresholds. Stimulation of the mucosa innervated by the SLN elicits reflex laryngeal closure, which is a brief closure of the true vocal folds.[15,16] The advantage of eliciting the laryngeal closure reflex is that it is an involuntary reflex and allows testing of patients with cognitive deficits who might not be able to reliably respond to psychophysical testing.[17,18] Because it is an involuntary reflex, the laryngeal closure reflex adds greater objectivity to LP sensory discrimination testing than psychophysical testing alone. The same air pulse stimulus was thus used to produce both a psychophysical response and an involuntary response in the subjects.

*Stimulus Delivery.* To measure the supraglottic and pharyngeal sensory level, a pressure- and duration-controlled pulse of air was delivered either to the anterior wall of the pyriform sinus or to the AE folds (the area innervated by the SLN). The air pulse, 50 milliseconds (ms) in duration, was varied in intensity from 0 to 15 mm Hg. It was delivered via an internal port located within a flexible fiber-optic telescope made for this purpose (Pentax Precision Instrument Corporation, Orangeburg, NY). The air pulse was obtained from an LP air pulse sensory stimulator developed for this purpose (Pentax Precision Instrument Corporation).

The flexible laryngoscope was inserted into the nasal passage, and the distal tip of the scope was advanced, under direct vision, until it was located approximately 2 mm from the test site. No anesthesia was utilized. The patient was then given a 1-minute rest period to adapt to the laryngoscope and prepare for testing. A masking sound was used to prevent the patient from using auditory cues during stimulus delivery to determine the presence of a stimulus.

*Psychophysical Testing.* With this technique, air pressure was varied according to the psychophysical technique of the ascending-descending method of limits, while the duration of the air puff was held constant at 50 ms. Prior to threshold determination, the patient was first provided a detailed description of apparatus and procedure. Next, air pulse stimuli were delivered to the cheek to demonstrate the phasic nature of the stimulus and the tapping or touch-like sensation that it evokes. The scope was then inserted as described above. To orient the subject to pharyngeal and laryngeal stimulation, we initially presented a suprathreshold stimulus to the test site in the laryngopharynx whose duration was 2 seconds rather than the phasic 50-ms stimulus used for testing. This stimulus was easily detected by the subjects. Subsequently, six blocks of stimulus administration trials were given. Each trial lasted for approximately 10 seconds, with a 10-second intertrial interval. The air pulse was administered after a verbal announcement of the beginning of the trial at a random time from 2 to 8 seconds within the 10-second trial interval. A detection response occurred when the subject raised his or her hand within 2 seconds of stimulus presentation. Three blocks of descending pressure stimulus presentations and three blocks of ascending pressure stimulus presentations were randomly ordered for each subject. Within a block, air puffs were presented in sequential steps of $7.5 \times 10^{-3}$ mm Hg: from subliminal to supraliminal for ascending blocks and from supraliminal to subliminal for descending blocks. A 30-second rest period separated the blocks. The mean of the lowest detected pressures from the six blocks was used as that subject's sensory threshold.

Two modifications to the ascending-descending method of limits were used to ensure accurate threshold determinations. First, catch trials were introduced in which a stimulus was not presented. This was accomplished by reducing stimulus intensity to zero but continuing to ask the patients if they felt the air pulse. The absence of a patient's response on these trials verified that they were responding to the air

TABLE 1. LARYNGOPHARYNGEAL SENSORY DISCRIMINATION TESTING FORM

Study ID Number: ______________________

Medical Record Number: ______________

Sex: _____ 0 = F 1 = M

Age: _____

Date: _____

Race/ethnicity: _____ 0 = white, NH; 1 = black, NH; 2 = Hispanic; 3 = other

Diagnosis: _____ 1 = stroke, 2 = dementia, 3 = other

HISTORY

Current diet (0 = No 1 = Yes): nothing by mouth: _____ nasogastric tube: _____ gastrostomy tube: _____

Liquid: _____ 0 = honey, 1 = nectar, 2 = thin, 3 = none

Solid: _____ 4 = puree, 5 = mechanical soft, 6 = regular, 7 = none

Dysphagia Solids: _____ 0 = No 1 = Yes

Liquids: _____ 0 = No 1 = Yes

Coughing while eating: _____ 0 = No 1 = Yes

Coughing while NOT eating: _____ 0 = No 1 = Yes

Weight loss: _____ 0 = No 1 = Yes

Throat numbness: _____ 0 = No 1 = Yes

LARYNGOPHARYNGEAL (LP) SENSORY TESTING

+: Positive response –: Negative response C: Catch trial LC: Laryngeal closure ASC: Ascending DES: Descending

Data (mm Hg)

| Right | | | | | | Left | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ASC1 | ASC2 | ASC3 | DES1 | DES2 | DES3 | ASC1 | ASC2 | ASC3 | DES1 | DES2 | DES3 |

PHYSICAL EXAMINATION

Vocal fold paralysis: _____ 0 = No 1 = Unilateral 2 = Bilateral

Pooling of secretions vallecula: _____ 0 = No 1 = Yes

pyriform sinus: _____ 0 = No 1 = Yes

Laryngeal penetration: _____ 0 = No 1 = Yes

Aspiration: _____ 0 = No 1 = Yes

SAFETY

Airway compromise: _____ 0 = No 1 = Yes

Epistaxis: _____ 0 = No 1 = Yes

Patient discomfort: _____ 0 = None 1 = Mild 2 = Moderate 3 = Severe

If your physician advised that you repeat this test, would you repeat this test? _____ 0 = No 1 = Yes

pulse stimulus and not to an extraneous cue such as the masking sound. Second, stimuli were presented by a single-blind procedure in which the examiner did not have knowledge of stimulus intensity. This was accomplished by having an associate working with the examiner set the stimulus intensity. In this way, the examiner did not know the starting subliminal stimulus intensity, the intensity of the largest supraliminal stimulus used, or on which trials the stimulus was not presented (catch trials). Moreover, the examiner did not know if the stimulation block consisted of air pulses presented in ascending or descending order. Data obtained for LP sensory testing were recorded on an LP sensory testing form

TABLE 2. PATIENT DATA ON SILENT LP SENSORY DEFICITS AFTER STROKE

| *Case* | *Sex* | *Age* | *CVA Site* | *LP Sensory Threshold* | *AP* |
|---|---|---|---|---|---|
| 1 | F | 59 | R Supra | Normal | No |
| 2 | M | 58 | R Supra | Normal | No |
| 3 | F | 77 | L Infra | Severe L | No |
| 4 | F | 65 | L Supra | Severe R, moderate L | No |
| 5 | F | 65 | R Supra | Normal | No |
| 6 | F | 63 | R & L Supra | Normal | No |
| 7 | M | 72 | L Infra | Severe R & L | Yes |
| 8 | M | 62 | R Supra | Normal | No |
| 9 | F | 71 | R Supra | Normal | No |
| 10 | F | 47 | L Supra & Infra | Severe R & L | No |
| 11 | F | 68 | R & L Supra & Infra | Severe R & L | No |
| 12 | F | 78 | R & L Supra | Normal | No |
| 13 | M | 52 | R & L Infra | Severe L | No |
| 14 | F | 45 | L Supra | Severe R | No |
| 15 | F | 80 | R Supra | Severe L, moderate R | No |
| 16 | M | 70 | L Supra & Infra | Severe R & L | Yes |
| 17 | M | 60 | R Supra | Severe L | No |
| 18 | F | 89 | R Supra | Severe R & L | No |

LP — laryngopharyngeal, CVA — cerebrovascular accident, AP — aspiration pneumonia, Supra — supratentorial, Infra — infratentorial.

(Table 1). Both the right and left sides of the pharynx and supraglottic larynx were studied.

*Reflex Laryngeal Closure.* The fiber-optic telescope was inserted into the nose, and unlike the psychophysical testing protocol, no verbal announcement of the beginning of a testing trial was made. Instead, the previously described three blocks of ascending and descending air pulse stimulus presentations were given, noting at what stimulation pressure transient vocal fold closure took place. Transient vocal fold closure was determined by direct visualization of vocal fold movement on a video monitor receiving a real-time image from the fiber-optic telescope. When the reflex is absent, the vocal folds fail to close after stimulation in this region. The type of vocal fold closure taking place in response to air pulse stimulation was a brief, rapid, nonrhythmic vocal fold adduction clearly distinguishable from the rhythmic vocal fold movement seen during normal respiration.[15-18] The mean of the lowest pressures from the six blocks that elicited brief laryngeal adduction was recorded as that subject's threshold.

*Outcome.* All patients were followed for up to 1 year from their original LP sensory test. No patient was lost to follow-up. Aspiration pneumonia was documented by cough, fever, decreased breath sounds, and the presence of an infiltrate on chest radiography.

## STATISTICAL ANALYSIS

The Wilcoxon score was used to test differences in threshold stimulus amplitudes between the affected side of the pharynx and the unaffected side. The Wilcoxon score was also used to test differences between thresholds of stroke patients and healthy controls. A Fisher's exact test was used to assess differences in outcome among the patients tested.

## RESULTS

Twelve women and 6 men with a mean age of 65.6 ± 11.5 years were studied. Twelve patients had supratentorial strokes, 3 patients had infratentorial strokes, and 3 patients had both supratentorial and infratentorial strokes (Table 2). All supratentorial strokes were cortical strokes involving the distribution of the middle cerebral artery. All the infratentorial strokes involved a portion of the lateral medullary distribution of the inferior cerebellar artery. There were no cognitive deficits in the stroke patients studied. The cortical stroke patients had transient contralateral upper and lower extremity hemiparesis that resolved by 6 months after the stroke. There were no cranial nerve deficits in the cortical stroke patients. The lateral medullary stroke patients had ipsilateral facial hypesthesia and contralateral body hypesthesia to temperature and pin prick. The gag reflex was intact and symmetric in all patients studied. There was no incidence of vocal fold paralysis or other cranial nerve deficits in the lateral medullary stroke group.

Previous work in healthy controls and in stroke patients with dysphagia has shown that 100% of healthy controls responded to air pulse stimulation of the laryngopharynx (either feeling the air pulse or having elicitation of the laryngeal adductor reflex) at

TABLE 3. MEANS AND STANDARD DEVIATIONS FOR LP SENSORY TESTING AND AGE OF PATIENT BY TYPE OF DEFICIT

| | *Normal* *(n = 7)* | *Unilateral Deficit* *(n = 6)* | *Bilateral Deficit* *(n = 5)* |
|---|---|---|---|
| LP sensory threshold (mm Hg) | 3.6 ± 0.8 | 6.4 ± 0.7 | 7.8 ± 0.4 |
| Age (y) | 65.1 ± 7.1 | 63.2 ± 13.7 | 69.2 ± 11.5 |

Differences between laryngopharyngeal (LP) sensory discrimination thresholds for all three groups were statistically significant at .05 level. No significant differences were found for average patient age between three groups.

air pulse pressures (APPs) of less than 4.0 mm Hg, while none of the dysphagic stroke patients responded below 4.0 mm Hg.[11] Stroke patients with dysphagia, if they responded at all to increasing APPs, only responded at a statistically significantly higher threshold, that is, above 6.0 mm Hg. The 5% of stroke patients with dysphagia that responded to air pulse stimulation between 4.0 and 6.0 mm Hg were considered to have moderate deficits. Therefore, LP sensory discrimination thresholds in this study were defined as either normal (<4.0 mm Hg APP), moderately impaired (4.0 to 6.0 mm Hg APP), or severely impaired (>6.0 mm Hg APP). Pressure application other than APP, pain threshold determination, and alteration in pH were not tested to stimulate the laryngopharynx.

Three groups of patients emerged from this study: those with normal LP sensory capacity, those with unilateral severe LP sensory deficits, and those with bilateral severe LP sensory deficits. The LP sensory discrimination was normal bilaterally in 7 of 18 patients (3.6 ± 0.8 mm Hg APP). There were 6 patients with unilateral severe LP sensory deficits (6.4 ± 0.7 mm Hg APP) — 2 of whom also had moderate LP sensory deficits on the contralateral side — and 5 patients with bilateral severe LP sensory deficits (7.8 ± 0.4 mm Hg APP). The differences between LP sensory discrimination thresholds for all three groups were statistically significant at the .05 level (Wilcoxon score; Table 3). No significant differences were found for average patient age between the three groups.

No LP sensory deficits were found in any of the age-matched controls. Among the stroke patients with unilateral deficits, sensory thresholds were severely elevated in all cases on the affected side compared with the unaffected side ($p < .01$, Wilcoxon score). Moreover, the sensory thresholds of the unaffected side were not significantly different from those of controls. Among the patients with bilateral severe deficits, the sensory thresholds were significantly different from those of the controls: 7.8 ± 0.4 mm Hg APP versus 2.5 ± 0.1 mm Hg APP ($p < .01$, Wilcoxon score).

Of the 12 patients with supratentorial strokes, 5 had LP sensory deficits. The LP sensory deficits that we identified in patients with unilateral supratentorial strokes (n = 4) were located contralateral to the side of the infarct. All 6 patients with any infratentorial strokes (3 infratentorial only, 3 supratentorial and infratentorial) had severe LP sensory deficits. The LP sensory deficits that we identified in patients with unilateral infratentorial strokes (n = 2) were located ipsilateral to the affected brain stem sites. Of the 5 patients with bilateral, severe LP sensory deficits, 4 had a combination of supratentorial and infratentorial strokes. No patients had vocal fold paralysis.

Outcome data were obtained on all patients in the study. There was no incidence of AP in the normal LP sensory group or in the group with unilateral severe LP sensory deficits. In the 5 patients with bilateral, severe LP sensory deficits, 2 out of 5 developed AP within 3 months of the LP sensory test, requiring readmission to the hospital ($p < .1$, Fisher's exact test). The underlying conditions of these patients did not change from the time of their sensory test. The 2 patients who developed AP both had bilateral, severe deficits of 8.0 mm Hg APP and were found to be the patients with the greatest sensory deficits. These 2 patients remained asymptomatic with regard to dysphagia symptoms at the time of their readmission to the hospital.

In addition to examining patients and controls for changes in the psychophysical threshold for detecting the air pulse, we also determined the threshold for eliciting reflex laryngeal closure. There was no statistically significant difference in LP sensory thresholds obtained by psychophysical testing versus reflex laryngeal closure testing.

## DISCUSSION

These results demonstrate, for the first time, that stroke patients without subjective or objective complaints of dysphagia have silent LP sensory deficits. In a silent LP sensory deficit, the patient has no complaints of dysphagia, yet has an LP sensory deficit. The concept of "silent aspiration" is different from silent LP sensory deficits. Silent aspiration, as defined in previous work, is a clinical situation in which the patient is complaining of dysphagia and a modified barium swallow (MBS) study is ordered. During the MBS study, penetration of food or saliva below the level of the true vocal folds takes place without cough or any other outward sign of difficulty by the patient.[14] The cause of silent aspiration is not known.

Whether silent LP sensory deficits are a contributing factor to silent aspiration is unclear, but they appear to be predictive of AP. A silent LP deficit may be even more insidious than silent aspiration. With silent LP deficits, neither the patient nor the patient's health care provider is aware of any problem with oral alimentation or has a sense of the potential danger until repeated episodes of pneumonia are confirmed. We propose that a portion of the 40,000 deaths a year that result from AP after a stroke occur in patients without dysphagia who have undetected sensory deficits. The recognition of these deficits early after a stroke could alert the clinician to the problem and warrant dietary modification to prevent AP.

Though our sample size is small, the results indicate that patients with infratentorial strokes are more likely to experience severe LP sensory deficits than patients with supratentorial strokes. An explanation for this clinical finding may be the location of the 10th nerve nuclei in the brain stem, which makes it vulnerable to infratentorial neurovascular injury.[19] Future work will be directed toward the development of more precise selection criteria for LP sensory discrimination testing in stroke patients.

Clinical bedside assessment of patients with swallowing disorders is unreliable.[4,20,21] Studies specifically looking at bedside clinical evaluation of patients with dysphagia and aspiration have shown that clinical assessment alone misses approximately 60% of those patients who go on to aspirate on MBS study.[20] One of the commonly employed aspects of the physical examination used to assess swallowing function at the bedside is the gag reflex, a method that has long been thought to serve as a gross means of LP sensory assessment.[22] However, the gag reflex test measures afferent activity in the 9th cranial nerve, not the SLN branch of the (vagus) 10th cranial nerve,[23,24] which provides sensation to the hypopharynx and larynx. It is not surprising, therefore, that assessment of the gag reflex does not give reliable prognostic information regarding the development of AP.[6,25]

The outcome data presented here strongly suggest that stroke patients with silent bilateral severe LP sensory deficits have the greatest risk of developing AP. Laryngopharyngeal sensory discrimination testing is an effective means of identifying these high-risk stroke patients who have no subjective dysphagia. Clinicians, therefore, can utilize the results of this objective measure to guide dietary management of their patients and reduce the risk of AP. We propose that patients who have had a stroke, whether or not they are complaining of dysphagia, may require an evaluation of their LP sensory capacity.

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## INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

The biannual meeting of the Interamerican Association of Pediatric Otorhinolaryngology will be held in Sao Paulo, Brazil, September 21-24, 1997. For information, contact Office of the Secretary, Tania Sih, MD, PhD, Rua Itapeva, 366, Suite 102, 01332-000, Sao Paulo SP, Brazil; telephone 55 11 283-4645 or 283-3396; fax 55 11 826-9652 or 542-6037.

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## PEDIATRIC OTORHINOLARYNGOLOGY

The Seventh International Congress of Pediatric Otorhinolaryngology will be held in Helsinki, Finland, June 7-10, 1998. For more information, contact Pekka Karma, MD, Chairman, or Hans Ramsay, MD, Secretary, ENT Department, Helsinki University Central Hospital, Haarmaninkatu 4 E, FIN-00290 Helsinki, Finland; telephone 358 9 4713019; fax 358 9 4715010; E-mail ramsay@hansram.pp.fi.

*Ann Otol Rhinol Laryngol* 106:1997

# CARTILAGINOUS TUMORS OF THE LARYNX: CLINICOPATHOLOGIC REVIEW OF 47 CASES

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We reviewed the clinical and pathologic features of 47 laryngeal cartilaginous tumors treated at the Mayo Clinic. This represents the largest reported series of these tumors. The patient group consisted of 36 men (77%) and 11 women (mean age, 63 years; range, 44 to 91 years). The tumors included 44 chondrosarcomas, all low-grade, and 3 chondromas. The overall 5-year survival was 90.1%, which did not differ significantly from the expected survival. Laryngectomy was performed as primary treatment for chondrosarcoma in 6 patients. Of the patients with chondrosarcoma, 40% had tumor recurrence or symptomatic tumor progression at an average of 4.5 years after diagnosis. Only 15 of the patients with chondrosarcoma (34%) required total laryngectomy. There were no metastases, and 4 patients died of local disease (all received treatment before 1960). At latest follow-up, 34 patients with chondrosarcoma (77%) were alive without disease or had died of other causes. We conclude that laryngeal chondrosarcoma can be treated with conservative surgery in most cases, both initially and for recurrent disease.

KEY WORDS — chondroma, chondrosarcoma, larynx, neoplasm, pathology, treatment.

## INTRODUCTION

Primary cartilaginous tumors of the larynx are rare neoplasms; the experience with these tumors at a single medical center is limited. Since the original descriptions of laryngeal chondroma in the early 19th century, more than 250 cases of chondromas and chondrosarcomas have been documented.[1] The largest previous series include 33 cases reported by Neel and Unni[2] from this institution and 31 cases reported by Hyams and Rabuzzi[3] from the Armed Forces Institute of Pathology. The appropriate surgical treatment of laryngeal cartilaginous tumors is still debated, and treatment recommendations have been based largely on cases collected from reviews of the literature.[4] Also, the natural history of these neoplasms and the potential prognostic factors have not been well delineated. To address these issues, we reviewed 47 patients with laryngeal cartilaginous tumors treated at the Mayo Clinic from 1910 to 1995.

## MATERIALS AND METHODS

The files of the Mayo Clinic were searched for potential cases of chondromas or chondrosarcomas of the larynx. Only patients who received treatment at the Mayo Clinic and for whom histologic slides were available for review were included in the study. The cases of several of these patients have been reported previously.[2,5] Criteria for the diagnosis of malignancy were based on those outlined for cartilaginous neoplasms of bone origin.[6] Clinical data were obtained by review of the medical records. All available histologic slides were reviewed by two pathologists (C.Y.I. and J.E.L.). Grading was based on previously published criteria.[7] Survival curves were estimated by the Kaplan-Meier method, and prognostic variables were analyzed by the logrank test.

## CLINICAL FEATURES

The patients included 36 men and 11 women who were 44 to 91 years old (mean, 63 years; Fig 1). Hoarseness was the most frequent presenting complaint (37 patients or 79%). Twenty-six patients (55%) described dyspnea, and 13 (28%) had dysphagia (Table 1). Unusual presentations included a previous clinical misdiagnosis of asthma in 2 patients and sleep apnea in another patient. Laryngeal chondrosarcoma was an incidental finding in 3 patients: a laryngeal mass was discovered on a radiologic examination for an unrelated problem in 2 patients, and in the other patient, a subglottic mass was found on a postoperative vocal cord check after biopsy of neck nodes for thyroid carcinoma. The mean duration of symptoms before diagnosis was 3.0 years (range, 1 month to 14 years).

Physical findings included a subglottic mass in 32 patients (68%), an external neck mass in 15 (32%), and a supraglottic mass or fullness in 9 (19%). The latter two findings were associated most frequently

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 4-5, 1996.

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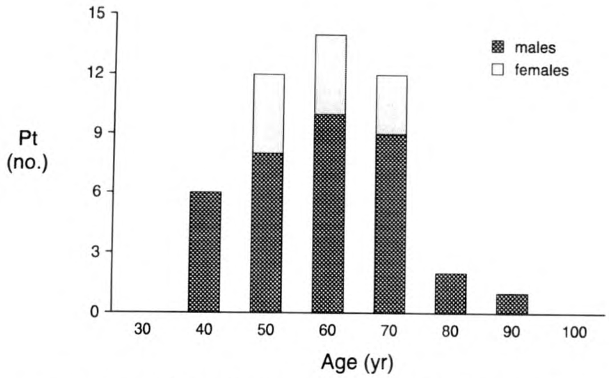

Fig 1. Age and sex distribution of 47 patients with laryngeal cartilaginous tumors.

with lesions arising in the thyroid cartilage. Cord paralysis was present in 23 patients (49%). Other physical findings were a glottic mass in 5 patients (11%; associated with subglottic involvement in 4 patients and a neck mass in 1) and hypopharyngeal swelling in 3 (6%).

The cricoid cartilage was the most frequent site of tumor origin (33 patients or 70%; Fig 2). Thirty tumors involved the posterior or lateral cricoid (or both), and 1 was circumferential. Only 2 tumors arose from the anterior cricoid. The thyroid cartilage was the site of origin of 12 tumors (26%). One chondroma arose in the epiglottis, and the cartilage of origin of 1 chondrosarcoma was unknown. In 7 cases (15%), large tumors extended to apparently involve adjacent cartilages, including the proximal trachea in 4 patients.

## PATHOLOGY

On pathologic review, we identified 44 chondrosarcomas and 3 chondromas. All chondromas were less than 1 cm in diameter. The chondromas were relatively hypocellular and composed of uniformly spaced chondrocytes that lacked nuclear atypia. Chondrosarcomas ranged in size from 1 to 13 cm (mean, 3.6 cm). Grossly, they formed relatively well-circumscribed but bulky masses that expanded and distorted the laryngeal framework (Fig 3). Thirty-three chondrosarcomas were grade 1, and 11 were grade 2. There were no high-grade (grade 3) tumors or dedifferentiated chondrosarcomas in the group. Chondrosarcomas were characterized by a lobulated growth pattern, with invasive growth of neoplastic lobules into adjacent soft tissue. Cellularity and nuclear atypia varied from area to area within a tumor. Neoplastic chondrocytes were distributed irregularly, resulting in a characteristic pattern of "cluster disarray." Cytologic features of chondrosarcoma included nuclear pleomorphism and hyperchromasia and the presence of binucleate forms (Fig 4). Mitotic activity, necrosis, and myxoid change were infrequent.

TABLE 1. SYMPTOMS AND SIGNS OF 47 PATIENTS WITH LARYNGEAL CARTILAGINOUS TUMORS

| *Symptom or Sign* | *Patients* *No.* | *%* |
|---|---|---|
| Hoarseness | 37 | 79 |
| Dyspnea* | 26 | 55 |
| Dysphagia | 13 | 28 |
| Neck mass | 10 | 21 |
| Foreign body sensation | 4 | 9 |
| None† | 3 | 6 |
| Cough | 2 | 4 |
| Pain | 1 | 2 |
| Sleep apnea | 1 | 2 |

*Includes 2 patients with previous misdiagnosis of asthma.
†Tumor was incidental finding in 3 patients.

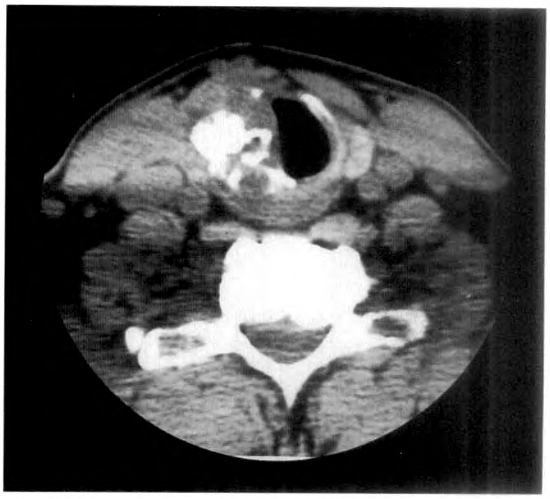

Fig 2. Computed tomographic scan of cricoid chondrosarcoma shows expansion of right lateral and posterior cricoid, with narrowing of airway and internal calcifications.

## TREATMENT

None of the patients received irradiation as primary treatment. Of the 3 patients with laryngeal chondroma, 2 had endoscopic removal and 1 had biopsy alone.

Forty-three patients with chondrosarcoma had their primary treatment at the Mayo Clinic. The type of operation depended on the location and extent of the tumor and the year of treatment. Surgical procedures could be categorized into five treatment groups: total laryngectomy, complete external removal, subtotal external removal, subtotal endoscopic removal, and biopsy. Total laryngectomy was performed as the initial operation in 6 patients (14%). A complete external removal was performed in 11 patients (26%). In these patients, the surgeon believed that the tumor could be removed entirely by an external approach while preserving most laryngeal function. This was

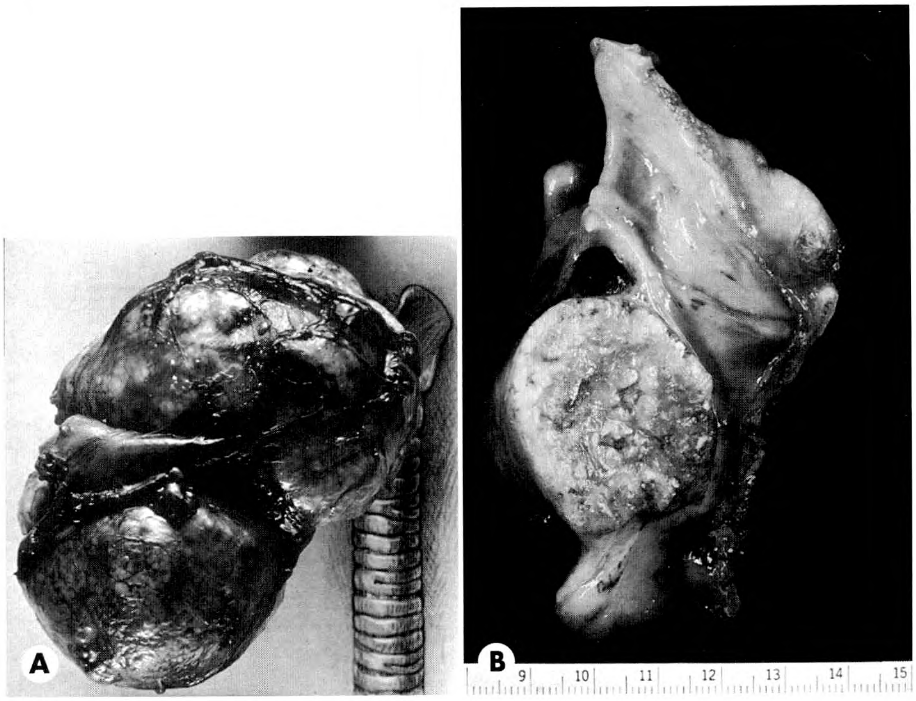

Fig 3. Surgical specimens. A) Grade 1 chondrosarcoma of thyroid cartilage; its greatest dimension was 13 cm. B) Chondrosarcoma of posterior cricoid. Cut surface shows relatively circumscribed mass that has lobulated growth pattern and translucent appearance characteristic of cartilaginous tumors.

accomplished either by laryngofissure for access or with an extralaryngeal subtotal resection of the larynx. Twenty-one patients (49%) had a subtotal external removal, in which some tumor was deliberately preserved. In this group, it was thought that removal of additional tumor would compromise laryngeal function and necessitate total laryngectomy. Subtotal endoscopic removal was performed in 3 patients (7%). Two patients (5%) had biopsy alone, without further treatment. Only 2 patients had postoperative irradiation, both from the subtotal external removal group.

An 80-year-old man with chondrosarcoma presented to the Mayo Clinic for a salvage operation. Ten years previously, he had undergone thyroid cartilage resection at another institution. A recurrent neck mass was discovered during the 6 months preceding treatment at the Mayo Clinic, where he underwent external removal (without thyrotomy) that was thought to be complete.

## FOLLOW-UP AND RESULTS

Two of the patients with laryngeal chondroma were lost to follow-up. The third patient is alive without disease 6 years after diagnosis.

For the 43 patients with primary treatment of their chondrosarcoma, the mean follow-up period was 10.6 years (range, 5 days to 31 years). No patient in this group was lost to follow-up. In examining recurrence or persistence according to treatment group, we found no patients in the primary total laryngectomy group with recurrence (Table 2). Of the 11 patients who had complete external removal, 3 had local recurrence (27%). One of these patients had no further treatment and was alive with disease at latest follow-up. Another patient had total laryngectomy 8 years after diagnosis, without further disease progression. The 1 other patient had surgical debulking 2.4 years after the original operation and had total laryngectomy for a second recurrence 6.3 years after the first treatment. This patient was alive without disease at latest follow-up.

In the subtotal external removal group, 14 of the 21 patients (67%) had local recurrence or obvious tumor progression, including both patients who had postoperative radiotherapy. Four of the patients with recurrence initially had total laryngectomy 1.4 to 5.8 years (mean, 3.1 years) after the subtotal procedure and subsequently were free of disease. Six of the 14 patients underwent debulking procedures after recurrence, 3 of whom subsequently had laryngectomy for a second recurrence 4.3 to 29.9 years after the first operation. After laryngectomy for a second recurrence, 2 of these 3 patients had additional local recurrence: 1 received no further treatment and was alive with disease at latest follow-up, and the other had removal of the neck mass and subsequently was free of disease. The third patient had no further recurrence after laryngectomy. Of the 3 other patients who had debulking after recurrence, 2 were without significant clinical disease at latest follow-up and 1 had died of an unknown cause after a second recurrence. The last 4 patients in this treatment group had recurrence or progression treated as follows: 1 patient received brachytherapy for first recurrence after

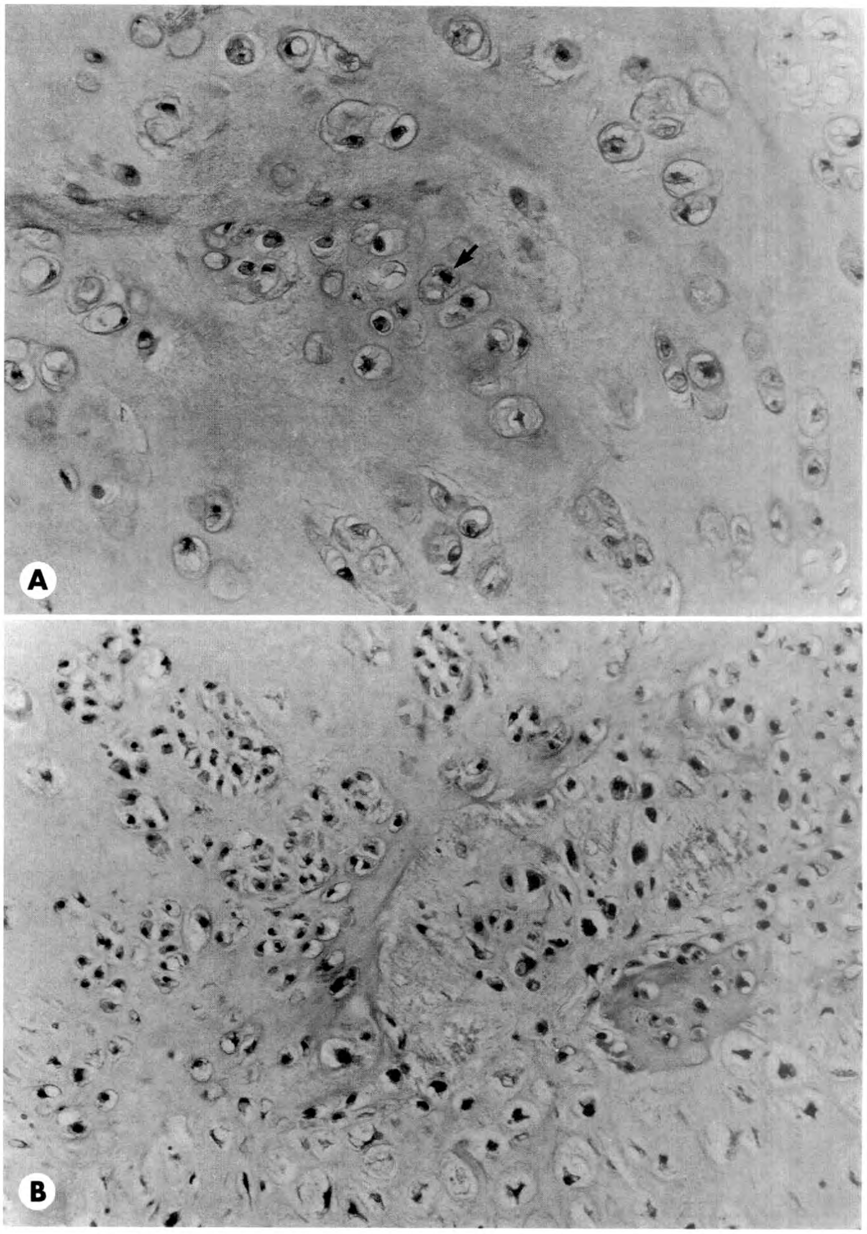

Fig 4. Chondrosarcoma (H & E, original ×50). A) Grade 1. Note irregular distribution of neoplastic chondrocytes, mild nuclear hyperchromasia and pleomorphism, and occasional binucleate cells (arrow). B) Grade 2, with areas of increased cellularity and greater degree of cytologic abnormality. Neoplastic cells form clusters of irregular size and distribution (cluster disarray).

subtotal removal and died of other causes; 2 had no additional treatment after recurrence (1 died of disease 7.5 years after presentation, and 1 was alive with disease at latest follow-up). Additional treatment for the 1 other patient with local recurrence was unknown, and the patient subsequently died of disease 2 years after diagnosis.

No patient who had endoscopic subtotal removal of a laryngeal chondrosarcoma had obvious clinical recurrence. Both patients who had biopsy alone had persistent disease and died of disease, 1.1 and 4.7 years after diagnosis. The single patient whose first treatment at Mayo was for recurrence was alive without disease at latest follow-up.

Information on quality of life status after the latest treatment was available for 35 patients in the primary treatment group for chondrosarcoma. Seven patients who did not have laryngectomy still required tracheotomy. Voice quality was assessed subjectively as good in 16 patients, fair in 15, poor in 3, and unknown in 1. Limitation of swallowing was noted in 2 patients, and restricted activity in 2 others. No patient complained of aspiration or had a feeding tube or gastrostomy.

Therefore, with the exception of the 2 patients who had biopsy only, 17 patients (40%) in the primary treatment group for chondrosarcoma had clinical recurrence or obvious tumor progression (Fig 5). First recurrence was at a mean of 4.3 years after initial treatment (range, 2 months to 14 years). Only 5 of these 17 patients had total laryngectomy at first recurrence. Twelve patients had a single recurrence,

TABLE 2. DISEASE PROGRESSION BY TREATMENT GROUP IN 43 PATIENTS WITH PRIMARY LARYNGEAL CHONDROSARCOMA

| *Initial Treatment* | *No. of Patients* | *Patients With Recurrence* | *Patients Dead of Disease* |
|---|---|---|---|
| Total laryngectomy | 6 | 0 | 0 |
| Total external removal | 11 | 3 | 0 |
| Subtotal external removal | 21 | 14 | 2 |
| Endoscopic removal | 3 | 0 | 0 |
| Biopsy only | 2 | 0* | 2 |
| Total | 43 | 17 (40%) | 4 (9%) |

*Both patients had persistent disease.

3 had two recurrences, and 2 were known to have three recurrences. At latest follow-up, 11 of the 17 patients (65%) were alive without disease or had died of other causes.

Altogether, 15 patients underwent laryngectomy at some time for chondrosarcoma. No patient had regional or distant metastases. Histologic dedifferentiation of chondrosarcoma did not develop in any of our patients. Four patients of the 43 (9%) died of local disease, all of whom had initial treatment before 1960. Two of these patients had biopsy only, without further therapy.

Overall survival for patients with chondrosarcoma was 90.1% at 5 years, 80.9% at 10 years, and 38.4% at 20 years. These figures were not significantly different from expected survival for a central North American control group matched for sex and date of birth (Fig 6). Disease-free survival was 67.6% at 5 years and 53.6% at 10 years. At latest follow-up, 21 patients were alive without disease, 4 were alive with disease, 4 had died of disease, 13 had died of other causes, and 1 had died of an unknown cause.

Statistical analysis was used to assess potential factors that could affect overall survival, disease recurrence, or the performance of a total laryngectomy for the 43 patients in the primary treatment group for chondrosarcoma. Only the presence of dyspnea had a deleterious effect on overall survival (p = .011). No other factor that was analyzed affected overall survival: age, sex, dysphagia, vocal cord paralysis, location (cricoid versus thyroid), size, grade, or type of operation. Disease recurrence was more frequent in younger patients (p = .067) and in those who had a subtotal surgical procedure rather than total external removal or laryngectomy (p = .052). Patients with dyspnea (p = .052) or cricoid tumors (p = .044) were more likely to undergo laryngectomy at some time in their treatment.

## DISCUSSION

Primary cartilaginous neoplasms of the larynx include chondromas and chondrosarcomas. True chondromas are probably rare; their differential diagnosis includes chondrometaplasia and low-grade chondrosarcoma.[8,9] Although most of the previously described laryngeal cartilaginous tumors originally were reported as chondromas,[1] many now are believed to represent chondrosarcomas.[8-10] We considered only 3 tumors in our series to be chondromas (6%). All of them were less than 1 cm in diameter, hypocellular, and cytologically bland.

Although laryngeal sarcomas are uncommon, chondrosarcoma is the most frequent. In a recent series of rare laryngeal tumors reported from the Mayo Clinic, 75% of laryngeal sarcomas were chondrosarcomas.[11] Chondrosarcomas typically affect men from 50 to 80 years old; we found a male-to-female ratio of 3.3 to 1 and a peak incidence in the seventh decade. Hoarseness is the most common presenting symptom and was found in 79% of our patients. Airway obstruction, dyspnea, and neck mass are other frequent complaints. In most patients the cricoid cartilage is the site of tumor origin. It has been postulated that the

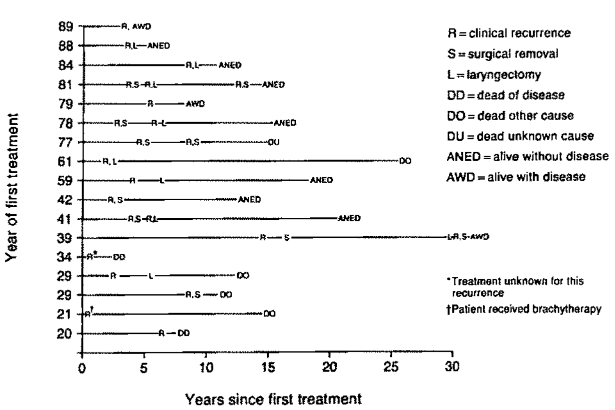

Fig 5. Time line of clinical follow-up in 17 patients with recurrent chondrosarcoma. Vertical axis depicts calendar year of first treatment for each patient (1920-1989), and horizontal axis represents years of follow-up since first treatment.

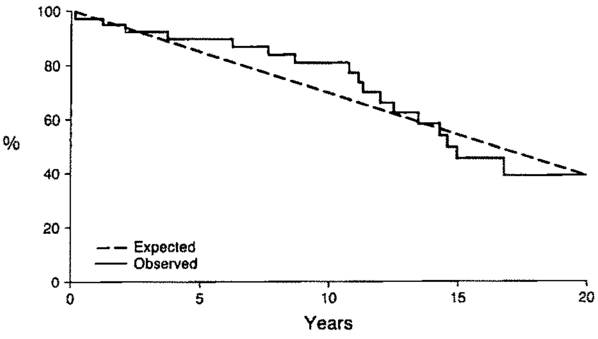

Fig 6. Overall survival for 43 patients with primary laryngeal chondrosarcoma. Difference from expected survival for control population matched for sex and date of birth was not significant.

development of chondrosarcoma may be linked to age-related ossification of the cricoid.[12,13]

The diagnosis of laryngeal chondrosarcoma is based on criteria for malignant cartilaginous tumors of bone origin, as described by Lichtenstein and Jaffe.[6] Similarly, grading of laryngeal tumors follows that for chondrosarcomas elsewhere in the body.[7,9] The majority of previously reported cases have been grade 1 or grade 2, as were all tumors in the present series. However, high-grade (grade 3) tumors have been described in the larynx,[14,15] as have several examples of dedifferentiated chondrosarcoma.[12,15-17] In bony sites, the diagnosis of dedifferentiated chondrosarcoma implies an ominous prognosis.[18] It appears that this also may be true for dedifferentiated tumors in the larynx, because metastases have been reported in several cases.[15]

The natural history of chondrosarcoma of the larynx is characterized primarily by indolent growth and potential for local recurrence. The recurrence rate in our series was 40% (17 of 43 patients) at a mean of 4.5 years after diagnosis. This is comparable to the overall recurrence rate of 37% reported by Hoffer et al[19] in a review of 84 cases from 12 recent studies.

Metastases have been reported in at least 20 cases of laryngeal chondrosarcoma documented in the literature,[15,20] although we did not find any in 43 cases with a mean follow-up of 10.5 years. This may partly reflect some bias in the reporting of more aggressive tumors; one could also speculate whether presumed metastatic disease without pathologic confirmation in several instances may represent another process. However, well-documented cases of metastasis from laryngeal chondrosarcoma have been reported, and it has been suggested that metastases are more frequent with high-grade tumors.[9,21,22] Death from disease is uncommon and usually results from uncontrolled local growth. Four of our patients (9%) died of disease. In all 4 of these patients, the diagnosis was made before 1960, and all of them had locally recurrent or persistent tumor; 2 had biopsy alone and 2 had local recurrence after initial subtotal removal. Lavertu and Tucker,[4] in their analysis of 46 cases reported in the literature, identified 6 deaths due to local and/or distant disease (13%).

The overall prognosis for chondrosarcoma of the larynx is more favorable than for chondrosarcoma arising in other sites of the head and neck or elsewhere in the body.[21,23-25] Indeed, our overall 5-year survival was 90.1%, which was not significantly different from the expected survival for a matched control group.

In our study, only the presence of dyspnea had a significant effect on overall survival ($p = .011$). The type of surgical treatment and age had bearing on recurrence, nearly achieving statistical significance ($p = .052$ and $p = .067$, respectively). Other authors have suggested a correlation between pathologic grade and recurrence,[2,21] an association that has been established for chondrosarcoma of bony origin.[26] We were unable to confirm this relationship. Furthermore, the recurrence rate in our series for chondrosarcomas of the thyroid cartilage was not statistically different from that for chondrosarcomas of cricoid origin, although a higher recurrence rate for thyroidal tumors was reported previously from our institution.[2]

Traditionally, neither radiotherapy nor chemotherapy has been considered to play a significant role in primary or adjuvant treatment of chondrosarcoma. However, some promising results have been achieved with the use of irradiation in chondrosarcoma of bone.[27] We could discern no beneficial effect in the small number of patients receiving postoperative irradiation in our study group.

The appropriate surgical treatment of laryngeal chondrosarcoma has been debated. We would emphasize several clinical and pathologic features of laryngeal chondrosarcomas that became evident during our review and that we believe are important considerations in treatment recommendations.

1. None of our patients had regional or distant metastases, and death from disease appears unlikely in the modern treatment era.

2. High-grade or dedifferentiated chondrosarcomas of the larynx are probably very rare, because we found no examples in the records of the Mayo Clinic.

3. Laryngeal chondrosarcomas are characterized by a slow growth rate, as manifested by prolonged duration of symptoms (mean, 3 years); lack of significant clinical recurrence in 10 of 24 patients who had subtotal procedures (21 external, 3 endoscopic); a mean of 4.7 years until further treatment was required in 11 patients with recurrence who initially had subtotal removal; and a mean of 6.0 years after first treatment until subsequent laryngectomy in 9 patients who had initial procedures less than total laryngectomy. This slow growth rate should be considered with respect to the mean age at diagnosis, which was 63 years.

Although most of our patients received treatment before the advent of modern imaging techniques, we consider radiologic documentation of the extent of the tumor essential in treatment planning. Currently, high-resolution computed tomography is the optimal modality for evaluating cartilaginous tumors of the larynx.[10,28,29]

We believe that laryngectomy rarely is required as initial treatment for laryngeal chondrosarcoma. An external approach generally is recommended, whether with an extralaryngeal procedure or laryngofissure (depending on the site of origin and the relationship of the tumor to native cartilage). The tumor and involved cartilage should be removed until a margin of grossly and microscopically normal cartilage is achieved, provided that in doing so, laryngeal function can be preserved. If laryngeal function would be compromised by complete tumor removal, subtotal removal is appropriate, with clinical follow-up and further surgical treatment as necessary. In our opinion, it is preferable to remove the tumor to the extent that some laryngeal function can be preserved (and require tracheotomy for breathing) than to perform total laryngectomy. Patients receiving this type of treatment can expect to have reasonable speech and near-normal swallowing. Because of the indolent growth rate of laryngeal chondrosarcomas, we would agree that recurrences often can be treated by additional conservative surgery.[4,14,23,30] Total laryngectomy should be reserved for the unusual examples of very extensive or high-grade tumors, or for multiple recurrences.

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*Ann Otol Rhinol Laryngol* 106:1997

# SUPERIOR LARYNGEAL NERVE BRAIN STEM EVOKED RESPONSE IN THE CAT

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The generator sources of each wave of the laryngeal brain stem evoked response (LBR) have yet to be precisely demonstrated, although this has been studied in several animal species. This study was carried out to record the near-field brain stem activity as well as the far-field brain stem activity in the cat under the same experimental setup, and to search for the generator sources of the waves. Under general anesthesia, the LBR tracings were recorded adjacent to and within the brain stem following direct electrical stimulation of the internal branch of the superior laryngeal nerve. Reproducible positive and negative waves were detected by the far- and near-field techniques. Mean latencies, configurations, and reproducibility of each wave were demonstrated. From these results, we speculate on the generator sources of each wave.

KEY WORDS — generator source, laryngeal brain stem evoked response, laryngeal reflex, superior laryngeal nerve.

## INTRODUCTION

The physiologic functions of the larynx can be classified into several systems such as the respiratory, circulatory, phonatory, and protective reflex systems. Although phylogenetically primitive, the protective reflex may be the most important in protecting the lung against entrance of anything but air. The larynx provides this protection through a reflex closure triggered by tactile receptors in the glottic and supraglottic mucosa, which evoke the reflex contraction of the laryngeal muscles. This reflex is a polysynaptic brain stem response.[1-3] When this reflex is exaggerated or hypersensitive, it may be responsible for disorders such as spasmodic dysphonia, idiopathic laryngospasm, reflex apnea, apnea of infancy, stuttering, sudden infant death syndrome, and so on.[3-9]

Historically, in 1976, Sasaki and Suzuki[10] and Suzuki and Sasaki[11] recorded the short latency responses from the recurrent laryngeal nerve (RLN) after electrical stimulation of the internal branch of the superior laryngeal nerve (SLN) in animal and human experiments. They reported that the laryngeal adductor reflex response or the glottic closure reflex does exist in humans. In 1987, Isogai et al[12] described a method in which averaged electrical activity was recorded simultaneously from the RLN and the brain stem following electrical stimulation of the SLN in paralyzed cats. They termed this far-field brain stem activity the laryngeal brain stem response (LBR). In 1989, Anonsen et al[13] repeated the experiment of Isogai et al and suggested optimum recording parameters. In 1992, Kim et al[14] also reported on LBR recording in cats. More recently, Cohen et al[15] reported a comparative analysis of the latencies of their study and previous studies in piglets, and Montalt et al[16] used rabbits as an experimental model.

The proposed pathway of this laryngeal reflex consists of an SLN stimulation followed by vagal afferents carrying the stimulus through the nodose ganglion to the brain stem, ie, the nucleus solitarius. Then the laryngeal adductor motor neurons are stimulated, probably in the nucleus ambiguus and reticular formation, resulting in vocal cord adduction and pulmonary protection.[7]

However, human application of the LBR is still not feasible, because it requires an operative procedure to expose the SLN and also requires complete muscular paralysis. Furthermore, the generator sources of the LBR have not been confirmed to date.[13,15] Therefore, if the reflex pathways can be electrophysiologically verified, there will be considerable progress in the study and management of the above-mentioned disorders that may be linked to aberrant laryngeal function.

There have been numerous reports about the generator sources of the LBR peaks. Isogai et al[12] compared the latencies of RLN reflex peaks and LBR peaks. Anonsen et al[13] and Cohen et al[15] speculated on the generator source by comparison of far-field latencies with prior near-field latencies.[17-22] How-

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Presented at the meeting of the American Laryngological Association, Orlando, Florida, May 4-5, 1996.

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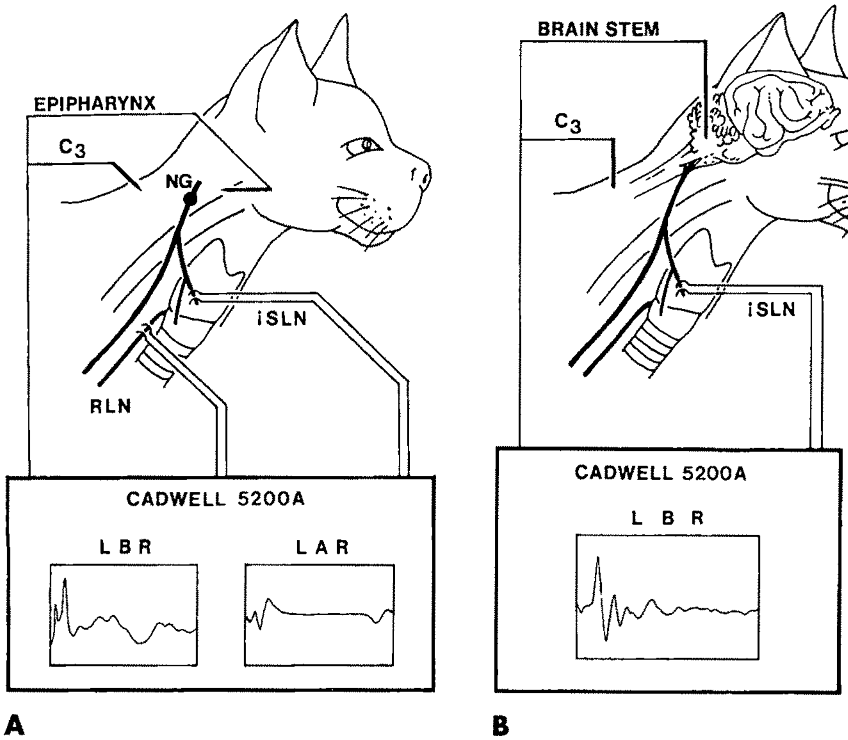

Fig 1. Experimental setup and animal preparation. A) For far-field technique. Laryngeal brain stem evoked response (LBR) was recorded from epipharynx and cervical (C3) electrodes after electrical stimulation of internal branch of superior laryngeal nerve (iSLN). LBR was also recorded after vagal neurectomy above nodose ganglion (NG). Laryngeal adductor reflex response (LAR) was recorded from recurrent laryngeal nerve (RLN). B) For near-field technique. LBR was recorded from nucleus solitarius or nucleus ambiguus region of brain stem and cervical (C3) electrodes after electrical stimulation of iSLN.

ever, no reports were made recording the far- and near-field potentials in the same experimental setup considering possible intraspecies variations.

In this context, we designed a study to compare the near-field with the far-field brain stem activity in the cat under the same experimental conditions. We also compared the waveform and latencies of LBR peaks recorded by the above two techniques. Finally, we speculated on the generator sources of LBR peaks through the comparison of our data with those of prior reports.

## MATERIALS AND METHODS

Twenty adult cats weighing 2.0 to 3.5 kg were used. They were divided into two groups of 10 each. One group was used for the far-field technique, and the other for the near-field technique, to record the brain stem evoked response. Pentobarbital sodium (25 mg/kg body weight) was intraperitoneally injected and a femoral venous catheter was introduced for maintaining anesthesia and for administration of drugs. The animals were endotracheally intubated and artificially ventilated (Polymed 201, SIMV Pulmolog, Draeger) at the rate of 26 times per minute, with the minute volume of 520 mL and fraction of inspired oxygen being set at 21% throughout the entire experimental procedure. The body temperature of the animals was maintained within a constant range (38°C to 39°C) with a heating pad.

In the supine position, the anterior neck skin was incised at midline from the level of the hyoid bone down to the suprasternal notch to expose the larynx and cervical trachea. Under the operating microscope, the internal branch of the right SLN was cut proximally to the entrance to the thyrohyoid membrane and bathed in mineral oil to prevent drying after desheathing the nerve for about 1 cm. The right RLN was identified in the tracheoesophageal groove and was cut proximally to the branching into the adductor and abductor branches. The nerve was preserved after the same preparation as performed on the internal branch of the SLN.

After the preparation just mentioned, the brain stem was exposed for the near-field recording of LBR peaks. The head of the animal was fixed in a stereotaxic frame (DJ-8001, Seoul, Korea) with a head holder and ear bars in the prone position. The occipital scalp was incised vertically at midline from the vertex to the upper cervical area. The exposed occipital bone was drilled out to make a bur hole of about 7 mm diameter to access the brain stem area. A part of the cerebellum was gently sucked out or retracted upward.

The preserved internal branch of the SLN was hooked around the insulated bipolar gold electrode for electrical stimulation.

To record the laryngeal adductor reflex response, an insulated bipolar gold electrode was hooked around the RLN (Fig 1A).

A stainless steel needle electrode was inserted through the soft palate perorally into the posterior

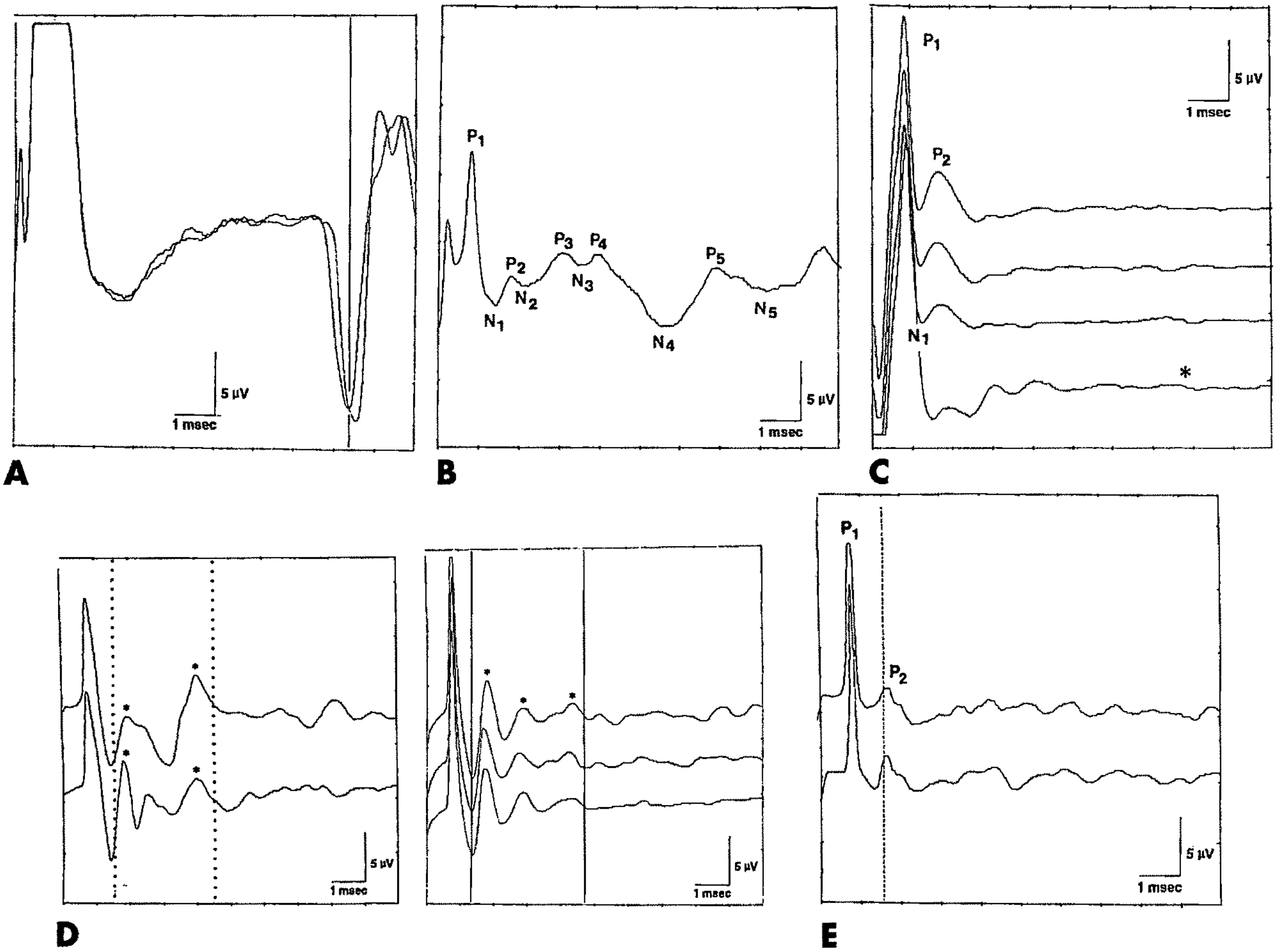

Fig 2. Results. A) Laryngeal adductor reflex response waveform recorded in recurrent laryngeal nerve after electrical stimulation of internal branch of superior laryngeal nerve. B) Far-field recording of laryngeal brain stem evoked response (LBR) waveform. C) Series of far-field recordings of LBR after supranodosal vagotomy (upper three recordings). Only P1, N1, and P2 waves were reproducible when compared with wave recorded before vagotomy (*). D) Series of near-field recordings of LBR at region of nucleus solitarius. Reproducible waves (*) were recorded in latency range of 1.59 to 4.49 milliseconds. E) Near-field recordings of LBR at region of nucleus ambiguus. Waves as whole were not reproducible, except earlier wave (P2) in some cases.

epipharyngeal wall to record the far-field LBR peaks. Needle electrodes were inserted in the paravertebral muscle at the level of the third cervical vertebra as a reference electrode and into the anterior neck muscle as a ground. In the far-field technique group, the variations of LBR peaks were also observed after sectioning the vagal trunk above and below the nodose ganglion with the same stimulus condition to try to ascertain the generator sources of the earlier waves of the LBR (Fig 1A).

For recording the near-field LBR peaks, an active electrode (insulated copper needle electrode, 10SNE, 200 × 40 mm) was inserted into certain areas of the brain stem, ie, the solitary tract nucleus and nucleus ambiguus, according to the coordinates of *A Stereotaxic Atlas of the Cat Brain.*[23] Reference and ground electrodes were inserted in the same manner as in the far-field technique (Fig 1B).

The animals were paralyzed with intravenous pancuronium bromide (0.1 mg/kg body weight), and electrical stimulation was started after more than 5 minutes to eliminate the possible effect of a muscle contraction artifact on the LBR recordings.

The SLN was stimulated with a Cadwell 5200A electrical stimulator and isolator using 0.1-millisecond (ms) square waves with a stimulus repetition of 400 stimuli, 3 times per second frequency, and 2 mA stimulus intensity. Stimulus artifact was minimized by alternating the polarity of the electrical stimulus. Responses were recorded by an averaging computer with a band pass of 320 to 1,000 Hz and a sensitivity of ±10 µV. The LBR and RLN responses were plotted with a Cadwell data recorder. Each stimulus condition was repeated more than twice to demonstrate response reproducibility.

## RESULTS

The laryngeal adductor response was taken from

TABLE 1. FAR-FIELD LARYNGEAL BRAIN STEM RESPONSE LATENCIES

| *Wave* | *No. of Responses* | *Latencies (ms)* *Mean* | *SD* | *Range* |
|---|---|---|---|---|
| P1 | 10 | 0.73 | 0.03 | 0.67 - 0.84 |
| N1 | 10 | 1.27 | 0.16 | 1.05 - 1.63 |
| P2 | 10 | 1.73 | 0.19 | 1.38 - 2.10 |
| N2 | 10 | 2.41 | 0.29 | 1.84 - 2.77 |
| P3 | 10 | 3.36 | 0.41 | 2.81 - 4.07 |
| N3 | 10 | 4.27 | 0.56 | 3.44 - 5.20 |
| P4 | 10 | 5.09 | 0.62 | 3.94 - 5.83 |
| N4 | 10 | 5.98 | 0.60 | 4.83 - 6.76 |
| P5 | 10 | 6.89 | 0.70 | 5.42 - 8.01 |
| N5 | 2 | 6.57 | 0.67 | 6.09 - 7.04 |

each cat of the far-field technique group. The mean (n = 10) latency was 8.61 ms, with an SD of 0.42, and the range was from 7.88 to 9.35 ms (Fig 2A).

With the far-field technique, stimulation of SLN evoked five positive and negative waves with reproducible latencies. The positive waves were labeled P1 through P5, and negative waves N1 through N5 in order of their appearance (Fig 2B). All the other waves showed reproducibility, but the N5 wave was only observed in 2 cats with reproducible latencies (Table 1).

In 4 cats of the far-field technique group, the LBR peaks were recorded after sectioning the vagal trunk above the nodose ganglion. The waves showed a random pattern with delayed latencies or disappearance and lost their reproducibility, except for P1, N1, and P2, which showed reproducibility compared to the previous waveforms obtained before transection of the vagal trunk (Table 2 and Fig 2C). Transection of the SLN eliminated all LBR activity except the triggering artifact.

With the near-field technique, reproducible waves were recorded in the latency range of 1.59 to 4.49 ms at the nucleus solitarius region. These waves corresponded to waves P2, N2, and P3 recorded by the far-field technique. Waves after N3 were not reproducible (Table 3 and Fig 2D). The near-field potentials were recorded at the nucleus ambiguus region. However, only P2 was reproducibly recorded from 5 cats, as all other waves were not reproducible (Table 4 and Fig 2E).

TABLE 2. LARYNGEAL BRAIN STEM RESPONSE LATENCIES AFTER SUPRANODOSAL VAGOTOMY

| *Wave* | *No. of Responses* | *Latencies (ms)* *Mean* | *SD* | *Range* |
|---|---|---|---|---|
| P1* | 9 | 0.73 | 0.03 | 0.67 - 0.75 |
| N1* | 9 | 1.21 | 0.16 | 1.00 - 1.51 |
| P2* | 9 | 2.33 | 0.74 | 1.59 - 3.52 |
| N2 | 6 | 3.19 | 0.57 | 2.73 - 4.11 |
| P3 | 7 | 3.89 | 0.47 | 3.15 - 4.49 |
| N3 | 5 | 4.64 | 0.58 | 3.65 - 5.20 |
| P4 | 4 | 5.48 | 1.05 | 3.94 - 6.34 |
| N4 | 2 | 5.90 | 0.45 | 5.58 - 6.21 |
| P5 | 1 | 6.59 | | |
| N5 | | | | |

*Reproducible waves.

TABLE 3. NEAR-FIELD LARYNGEAL BRAIN STEM RESPONSE LATENCIES RECORDED AT NUCLEUS SOLITARIUS REGION

| *Wave* | *No. of Responses* | *Latencies (ms)* *Mean* | *SD* | *Range* |
|---|---|---|---|---|
| P1 | 12 | 0.76 | 0.02 | 0.75 - 0.79 |
| N1 | 11 | 1.21 | 0.15 | 1.00 - 1.38 |
| P2 | 13 | 1.71 | 0.12 | 1.59 - 1.97 |
| N2 | 8 | 2.38 | 0.31 | 1.93 - 2.73 |
| P3 | 12 | 3.14 | 0.54 | 2.80 - 3.90 |
| N3 | 3 | NR | | |
| P4 | 3 | NR | | |
| N4 | 3 | NR | | |
| P5 | 3 | NR | | |
| N5 | 2 | NR | | |

NR — not reproducible.

## DISCUSSION

Laryngeal reflexes play an important role in several physiologic functions such as respiratory, circulatory, and phonatory controls and pulmonary protection from aspiration. Chemical or mechanical stimulation of the supraglottic mucosa or direct electrical stimulation of the SLN may result in several responses. One of them is the laryngeal adductor reflex response (LAR), which concerns glottic closure.[10,11] The others are centrally mediated apnea, respiratory rate changes, hemodynamic or cardiovascular changes, and swallowing, which can be catego-

TABLE 4. NEAR-FIELD LARYNGEAL BRAIN STEM RESPONSE LATENCIES RECORDED AT NUCLEUS AMBIGUUS REGION

| *Wave* | *No. of Responses* | *Latencies (ms)* *Mean* | *SD* | *Range* |
|---|---|---|---|---|
| P1 | 5 | 0.76 | 0.02 | 0.75 - 0.79 |
| N1 | 2 | 1.38 | 0.00 | 1.38 - 1.38 |
| P2 | 5 | 1.80 | 0.12 | 1.68 - 1.93 |
| N2 | 1 | NR | | |
| P3 | 2 | NR | | |
| N3 | 2 | NR | | |
| P4 | 2 | NR | | |
| N4 | 1 | NR | | |
| P5 | 1 | NR | | |
| N5 | 2 | NR | | |

NR — not reproducible.

TABLE 5. COMPARISONS OF LATENCIES OF LARYNGEAL BRAIN STEM EVOKED RESPONSE

| *This Study* | *Isogai et al*[12] *(1987)* | *Anonsen et al*[13] *(1989)* | *Cohen et al*[15] *(1993)* | *Generator Source in Near-Field Data* |
|---|---|---|---|---|
| P1: 0.73 | P1: 0.5 - 1.0 | P0: 0.78 | P1: 1.24 | Ipsilateral vagus nerve 1.1 ± 0.9 |
| N1: 1.27 | N1: 1.2 - 1.4 | N0: 1.10 | N1: 1.82 | (Lucier and Sessle[21]) |
| P2: 1.73 | P2: 1.6 - 2.3 | P1: 1.17 | | |
| P2: 1.73 | P2: 1.6 - 2.3 | P2: 2.37 | P2: 2.52 | Nucleus solitarius 1.3 - 3.5 (2 - 4) |
| N2: 2.41 | N2: 2.4 - 2.7 | N2: 2.76 | N2: 3.05 | (Porter,[17] Biscoe and Sampson,[18] |
| P3: 3.36 | P3: 2.8 - 3.2 | P3: 3.39 | P3: 3.37 | Sessle[19]) |
| N3: 4.27 | P4: variable | P4: 4.96 | N3: 4.71 | Nucleus ambiguus 5.0 - 10.0 (5.0 - 8.0) |
| P4: 5.09 | N4: 5.0 - 5.5 | N4: 5.52 | P4: 4.97 | (Porter,[17] Delgado-Garcia et al[22]) |
| N4: 5.98 | | | N4: 5.47 | |
| P5: 6.89 | | | P5: 6.25 | |
| P5: 6.89 | | P5: 6.65 | N5: 6.86 | Reticular formation? 7.0 (Sessle[19]) |
| | | | P6: 7.16 | |
| | | | N6: 7.89 | |

Data are in milliseconds.

rized as laryngeal chemoreflexes (LCRs).[9,24-26]

Changes of the laryngeal reflexes according to the external stimuli have been studied in various experimental models, including humans.[4,27-30] Suzuki and Sasaki[31] reported in their study of the effect of various sensory stimuli on reflex laryngeal adduction that SLN produces the least attenuation in its evoked responses on repetitive stimulation. Therefore, if an objective recording of an evoked brain stem response after stimulation of SLN is possible, it will be a valuable index for understanding of the pathophysiology of abnormal laryngeal reflexes. Like the auditory brain stem evoked response used in the diagnosis of patients with hearing loss, the LBR can be applied as an additional diagnostic tool in laryngeal disorders. The proposed pathway of this reflex is that the SLN stimulation is carried via vagal afferents through the nodose ganglion to the brain stem (nucleus solitarius). Then the laryngeal adductor motor neurons are stimulated, probably in the nucleus ambiguus and reticular formation.[7,18,20,21,32-36]

The electrode setting used in this study was modified by the authors from the previously reported methods to obtain the optimal responses. The SLN was stimulated with the same optimal suprathreshold intensity we used in our previous study[14] and on the basis that an increase of stimulus intensity does not affect the latency significantly, as reported by Anonsen et al.[13]

The reproducibility of the responses was verified by repeating each experiment several times. Pancuronium bromide was used throughout the entire procedure to avoid possible muscular contraction artifact. Montalt et al[16] stated that adequate sedation may eliminate bothersome muscular artifacts without the need of curarinic drugs or mechanical ventilation. However, curarinic drugs have been commonly used until now.

Each wave peak was numbered in the order of appearance. There were differences of recorded wave peaks according to the authors and experimental animals. Anonsen et al[13] recorded six positive and five negative waves in cats, and Cohen et al[15] recorded six positive and negative waves in piglets. Montalt et al[16] recorded five positive and four negative waves in rabbits. These differences can be explained by several possible factors. The electrode position can alter the waveform morphology and polarity. And the differences in filter settings, particularly the low frequency, can affect both waveform morphology and latency.[13] Regarding the effect of changes in PO2 and PCO2, Isogai et al[12] reported that they can change latencies and amplitudes of evoked responses in the cat. Anonsen et al[13] even stated that the laryngeal system could behave in an all-or-none fashion, as it is a protective one. But Cohen et al[15] stated that the LBR is unresponsive to changes in arterial oxygen and carbon dioxide concentrations in piglets. So, the results may vary according to the experimental animal. Additionally, the type and level of anesthesia, age, sex of the animal, and temperature can be possible explanations.

Transection of the vagal trunk above the nodose ganglion eliminated all waves except P1, N1, and P2. Transection of the SLN proximal to the stimulating electrode eliminated all waves except the triggering artifact. These results suggest that the earlier waves are generated by the ipsilateral vagus nerve and that the waveforms obtained in this study can be regarded as neural activity evoked by SLN stimulation. Other reasons are as follows. First, the initial peak occurred after SLN stimulation in each observation. Second, consistent responses to stimuli of alternating polarity suggest that these waves are nonrandom activities triggered by SLN stimulation. Third, transection of the SLN abolished all the peaks except stimulus artifact. Finally, that the waveforms persisted under complete muscular paralysis suggests a neurogenic origin.[15]

Regarding the generator sources of LBR peaks, Isogai et al[12] correlated the direction of potentials to their order of appearance, and speculation was done by comparing obtained data with prior near-field data.[13,15] However, although the prior near-field data are reliable, they were not obtained under the same experimental conditions as each of the far-field techniques. So, we recorded far- and near-field potentials under the same conditions to reduce possible error in an individual animal of the same species. Of course, interindividual differences were present in this study, which are thought to have originated from differences in the sensitivity of the electrodes to each of the wave peaks. Also, the complexity of the reflex response itself, which can be affected by many external factors, may be another explanation.[15] These differences may be further minimized when the far-field and near-field recordings are obtained within the same animal preparation (which is our next study).

A comparison of far-field data in this study with data from prior far- and near-field reports following electrical stimulation of the SLN in various animal models is listed in Table 5.[12,13,15,17-19,21,22]

The locations of the brain stem nuclei were determined by three-dimensional coordinates based on the interaural line. Although the location was precisely calculated, the head size difference of each animal makes the exact insertion of electrodes into the brain stem nuclei uncertain. To compensate for this possible error, the coordinates of the stereotaxic frame were recalibrated for each animal, and animals within a 2.0- to 3.5-kg body weight range were chosen to reduce the head size difference. To insert the electrodes into the correct location, the obex of the fourth ventricle rather than the interaural line is recommended as a base for calculation of coordinates.[21,22,33]

At the nucleus solitarius, the electrode was inserted into five locations selected at 0.5-mm intervals. After repetitive recording of the evoked responses, mainly the earlier waves were reproducibly recorded. Their latency range was 1.59 to 4.49 ms, which corresponded with the P2, N2, and P3 waves of the far-field potentials of this study. Waves beyond N3 showed no reproducibility, which suggests that the above three waves originated from the nucleus solitarius.

At the nucleus ambiguus, an electrode was inserted into four locations selected at 0.5-mm intervals. Almost all the waves lost their reproducibility. Only the P2 wave was detected in 5 out of 10 animals, which made the speculation of the generator source impossible at this nucleus. This may be due to the difficulty of exactly placing the electrode into the small and deeply located nucleus ambiguus. Although not performed in this study, the exact location of the electrodes may be verified more precisely through morphologic assessment.

The relationships between the LBR peaks and generator sources, however, are only suggestive, because firm conclusions regarding the anatomic origins of the LBR peaks would require lesional studies or stereotaxic recordings from generators along the reflex pathway.[15] For these lesional studies, the central projections of the afferent fibers of the SLN should be studied.

The intraoperative LBR tracing was reported by Yamashita[37] in a total laryngectomy patient in whom the internal branch of the SLN was stimulated by a 2.0-Hz square wave (stimulus intensity 1.4 mA, interval 0.2 ms) with a pair of monopolar electrodes. He recorded the waves averaged 200 times from the forehead surface electrodes. He also reported the brain stem response after stimulating the SLN area transcutaneously with hooked wire electrodes. Although his work showed the possibility of using transcutaneous stimulation of SLN, this method is not commonly used to date.[38]

The generator sources of the waves can also be speculated on from an intracranial lesional experiment in which the characteristics of the waves can be compared before and after a surgical transection of each brain stem area. And we can suggest another semi-noninvasive method in which the SLN is preserved and the supraglottic mucosa innervated by the SLN is stimulated mechanically, as in the recording of somatosensory response.[39] For this, a mechanical stimulator such as an air pump should be devised that enables repetitive, pulsed stimulation. Therefore, if the method is to be adapted for use in humans, an attempt should be made to characterize the LBR response, and furthermore, a method of completely noninvasive stimulation and recording should be devised.

In comparing the laryngeal reflexes of various animals to those of humans, dogs have been reported to have a more human-like laryngeal adductor reflex than cats.[10,40] Therefore, in the future, these kinds of experiments should be performed on the canine model, which is very similar in anatomy and physiology to humans. And with the development of noninvasive stimulation methods and characterization of each LBR peak, the LBR may be a definitive tool for the diagnosis and treatment of laryngeal disorders.

## CONCLUSIONS

The LBRs were recorded in the cat adjacent to and within the brain stem following direct electrical stimulation of the internal branch of the SLN, and the following results were obtained:

1. The mean latency of the laryngeal adductor reflex response was 8.61 ms.
2. By the far-field technique, a total of five reproducible positive and negative waves were detected, with mean latencies ranging from 0.73 to 6.57 ms.
3. After supranodosal vagotomy, only waves P1, N1, and P2 showed reproducibility.
4. After sectioning of the SLN, no waves were reproducible except triggering artifact.
5. By the near-field technique, waves of the latency range 1.59 to 4.49 ms were recorded around the nucleus solitarius region.
6. No reproducible waves were recorded around the nucleus ambiguus region.

These results suggest that waves P1, N1, and P2 of far-field recordings originated from the vagus nerve. And waves P2, N2, and P3 are thought to originate from the nucleus solitarius, from comparison of the far- and near-field data in this study. Through a comparison of the far-field data in this study with prior near-field reports, it was speculated that the nucleus ambiguus was the generator source of waves P4, N4, and P5.

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## FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

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*Ann Otol Rhinol Laryngol* 106:1997

# CHRONIC MAXILLARY ATELECTASIS

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Chronic maxillary atelectasis is a descriptive term that refers to a persistent decrease in the sinus volume of the maxilla from inward bowing of the antral walls. Case reports with comparable clinical presentations have appeared sporadically in the literature; however, this disorder has remained poorly defined. The purpose of this study is to provide a formal definition of this condition by the establishment of diagnostic and staging criteria. A 10-year case analysis identified 22 adults, and a review of the literature revealed another 25. The average age at presentation in our study was 38.3 years. Most patients were symptomatic, and some presented with diplopia and hypoglobus. Inward bowing of the antral wall(s) and persistent opacification on computed tomography made the diagnosis. Chronic maxillary atelectasis was separated into three stages according to the degree of wall deformation. While most patients were symptomatic, a past history of absent or mild symptoms referable to the nose and sinuses was encountered more often in those patients with osseous wall deformation ($p = .041$). Mild or absent symptoms at the time of diagnosis should not be considered a negative risk factor for the development of facial deformity, especially if the sinus has features consistent with complete pneumatization. A middle meatal antrostomy appears to relatively safely correct the sinus problem, while orbital floor reconstruction for hypoglobus, found in stage III of the disease, can be accomplished effectively via a transconjunctival approach using a combination of bone allograft and porous polyethylene sheets.

KEY WORDS — atelectasis, diagnosis, enophthalmos, hypoglobus, maxillary sinus, staging, treatment.

## INTRODUCTION

In 1964, Montgomery[1] reported 2 cases of enophthalmos and maxillary sinus opacification. These patients had collapsed bony walls and tenacious mucus filling the maxillary antrum. Case reports with comparable clinical presentations have appeared sporadically in the literature since that time.[2-10] Chronic maxillary atelectasis (CMA) is a descriptive term that refers to a persistent decrease in the sinus volume of the maxilla from inward bowing of the antral walls. The purpose of this study is to formally define this condition by the establishment of diagnostic and staging criteria.

Diagnostic criteria and a staging system were devised from an analysis of data from 22 new cases identified at this institution over a 10-year period. A review of the literature revealed an additional 25 cases that met the diagnostic criteria for CMA. From the literature review the average age of the patient at the time of diagnosis was 37.1 years, and there was no significant gender predominance. Symptoms referable to the nose and sinuses were present in 32% of patients from the case reports reviewed, and they tended to be mild to moderate in degree. In most of the case reports, patients had orbital and facial deformities with disease mainly limited to the maxillary sinus and middle meatus.

The cause of CMA is controversial.[2,3,6,7,11] Some consider CMA to be an unusual sequela of ostiomeatal complex mucosal disease in patients with chronic rhinosinusitis.[4-9] Antonelli et al[3] have theorized that negative pressure within the maxillary sinus may play an important role in the pathophysiology of this disorder. The medial infundibular wall (MIW) has not been previously implicated as playing an active role in the establishment of CMA. In this study, the possibility of valvular occlusion by the MIW is explored. Three cases are presented to highlight each stage of the disorder, and treatment options are discussed.

## METHODS

A 10-year retrospective search was done to identify those patients at the Massachusetts Eye and Ear

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Presented to the Eastern Section of the American Laryngological, Rhinological and Otological Society, Inc, Pittsburgh, Pennsylvania, January 27, 1995.

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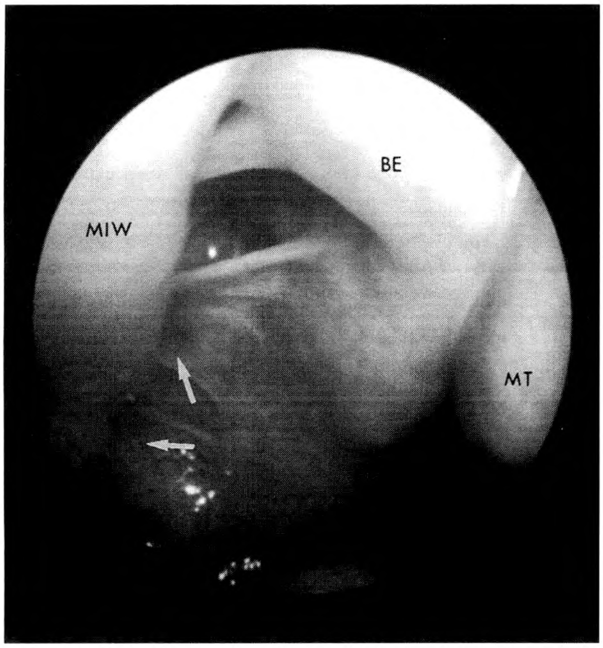

Fig 1. (Case 6) Rigid rhinoscopy demonstrates lateralized medial infundibular wall (MIW) with marked retraction of fontanel (arrows). Middle turbinate (MT) and bulla ethmoidalis (BE) can also be seen.

TABLE 1. STAGING OF CHRONIC MAXILLARY ATELECTASIS

Stage I (membranous deformity)
Lateralized maxillary fontanel
Stage II (bony deformity)
Inward bowing of one or more osseous walls (anterior, superior, and/or posterolateral bony walls) of maxillary antrum
Stage III (clinical deformity)
Enophthalmos, hypoglobus, and/or midfacial deformity

Infirmary who met the following criteria for the diagnosis of CMA:

1. Sinus opacification on repeat x-ray (plain or computed tomography [CT]) of at least 3 months' duration and/or the intraoperative finding of tenacious mucus secretions filling the antrum.
2. Lateral displacement of the MIW and fontanel as documented on clinical examination, endoscopic exploration (Fig 1), or CT, with or without inward bowing of the osseous walls.

The medical records and operative reports were reviewed. Nasal congestion, sneezing, and nonpurulent rhinorrhea were categorized as mild sinonasal symptoms, whereas pain and purulent nasal drainage were considered moderate to severe sinonasal symptoms. Radiographic studies and histopathologic slides were carefully analyzed. Patients with a previous history of maxillary sinus surgery, maxillary trauma, tumor mass within or adjacent to the maxillary antrum, or an obvious congenital abnormality were excluded from the study.

Chronic maxillary atelectasis was conveniently separated into three stages (Table 1) according to the degree of wall collapse. In stage I, the fontanel and MIW were laterally positioned. In stage II, one or more osseous walls of the antrum bowed inward as well. In stage III, marked deformation of the antral walls manifested clinically as enophthalmos, hypoglobus, and/or midfacial depression.

A literature review from 1960 to the present was done to identify cases of CMA according to the same criteria used for making the diagnosis in our case series (Table 2[1-9,12-15]). Several subject headings and title words were used to conduct a comprehensive review and these included enophthalmos, maxillary hypoplasia, malar deformity, sinus atelectasis, antral opacification, and mucocele. Those cases that did not meet minimum criteria through reports of x-ray re-

TABLE 2. SUMMARY OF LITERATURE REVIEW ON CHRONIC MAXILLARY ATELECTASIS

| *Authors* | *Mean Age (y)* | *m/n** | *Gender (M:F)* | *Sinus-Related Symptoms* | *Side of Involvement* | *Stage* |
|---|---|---|---|---|---|---|
| Bierny and Dryden[15] | 37 | 1/1 | 0:1 | 1/1 | L | III |
| Hanna et al[14] | 27 | 1/2 | 0:1 | 1/1 | R | I |
| Goldhammer et al[13] | 26 | 1/1 | 0:1 | 0/1 | R | III |
| Montgomery[1] | 34.5 | 2/2 | 2:0 | 0/2 | R 1, L 1 | III |
| Blackwell et al[2] | 44.3 | 3/3 | 1:2 | 2/3 | R 3, L 0 | III |
| Antonelli et al[3] | 48.5 | 2/2 | 2:0 | 0/2 | R 1, L 1 | III |
| Furin et al[4] | 32.0 | 2/2 | 0:2 | 2/2 | R 0, L 2 | II |
| Wilkins and Kulwin[5] | 36.3 | 4/5 | 1:3 | 1/4 | R 2, L 2 | III |
| Wesley et al[6] | 27 | 1/1 | 0:1 | 1/1 | L | III |
| Traustason and Feldon[7] | 34 | 1/1 | 1:0 | 0/1 | R | III |
| Martelli et al[8] | 45.5 | 2/5 | 1:1 | 0/2 | R 2, L 0 | III |
| Kaltreider and Dortzbach[12] | 29 | 1/4 | 1:0 | 0/1 | R | III |
| Soparker et al[9] | 37.0 | 4/14 | 2:2 | 0/4 | R 4, L 0 | III |

*m — patients who met diagnostic criteria for chronic maxillary atelectasis, n — total number of patients in study.

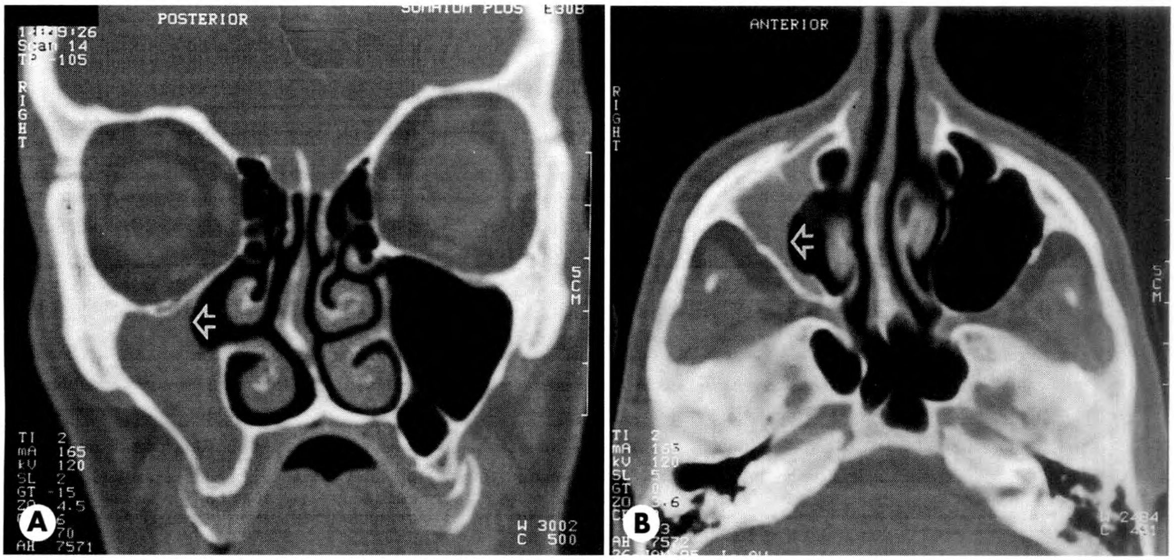

Fig 2. (Case 16) A) Coronal and B) axial computed tomograms of patient with stage I chronic maxillary atelectasis (CMA). Arrows — retracted MIW and fontanel.

sults, physical examination, and intraoperative findings were excluded from the review. The literature review identified several cases that met the diagnostic criteria for CMA; however, not all patients within a single study group met the criteria, and only those that did were included in this study.[2-10,12-14]

Data were compiled and analyzed with Microsoft Excel spreadsheets and Access database software (Microsoft Inc). Statistical analysis was performed by the SAS System (SAS Institute Inc, Cary, NC).

## CASE REPORTS

*Case 16.* A 38-year-old woman presented with headaches and persistent nasal blockage. Her symptoms had occurred intermittently for the past several years. The headaches were described as a bilateral dull periorbital pain. She denied any history of allergies. There was a large right septal deviation that limited the intranasal endoscopic examination. She did not have a malar depression deformity, enophthalmos, or hypoglobus. A CT scan of the paranasal sinuses showed complete right maxillary sinus opacification with fontanel retraction and no evidence of osseous wall collapse (Figs 2 and 3). She was found to have stage I CMA and underwent a septoplasty and middle meatal antrostomy (MMA). The MIW was positioned laterally and occluded an unremarkable maxillary sinus ostium (MSO). The antrum was filled with thick gray secretions and the histopathology showed chronic inflammation with mucus retention. The patient did well postoperatively, with improvement of her symptoms.

*Case 5.* A 38-year-old woman presented with intermittent right facial pressure sensation and nasal

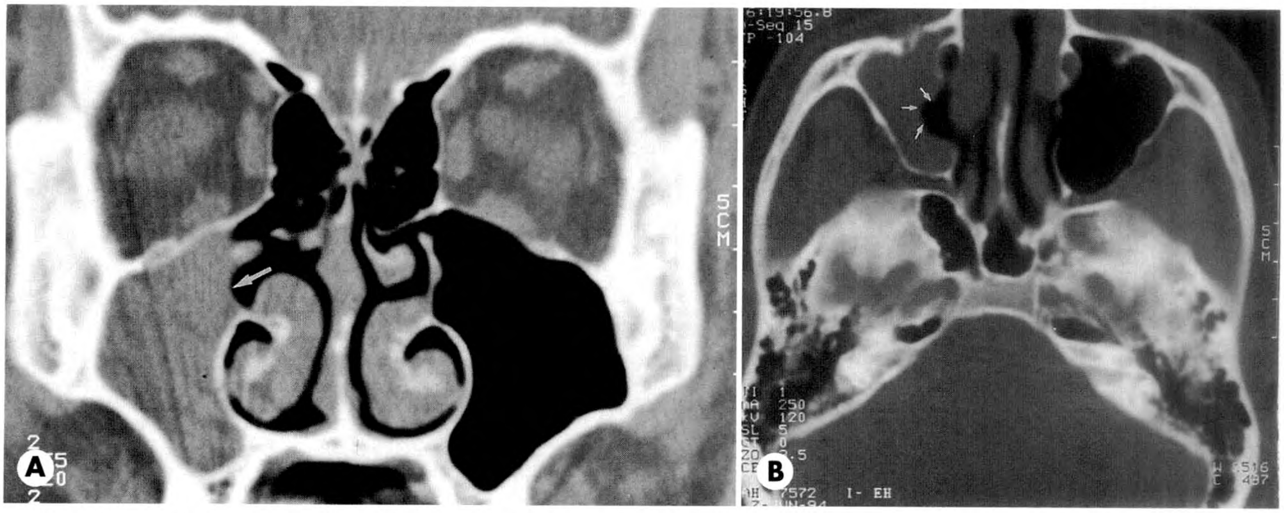

Fig 3. (Case 6) A) Coronal and B) axial computed tomograms of patient with stage I CMA. Arrows — retracted MIW and fontanel.

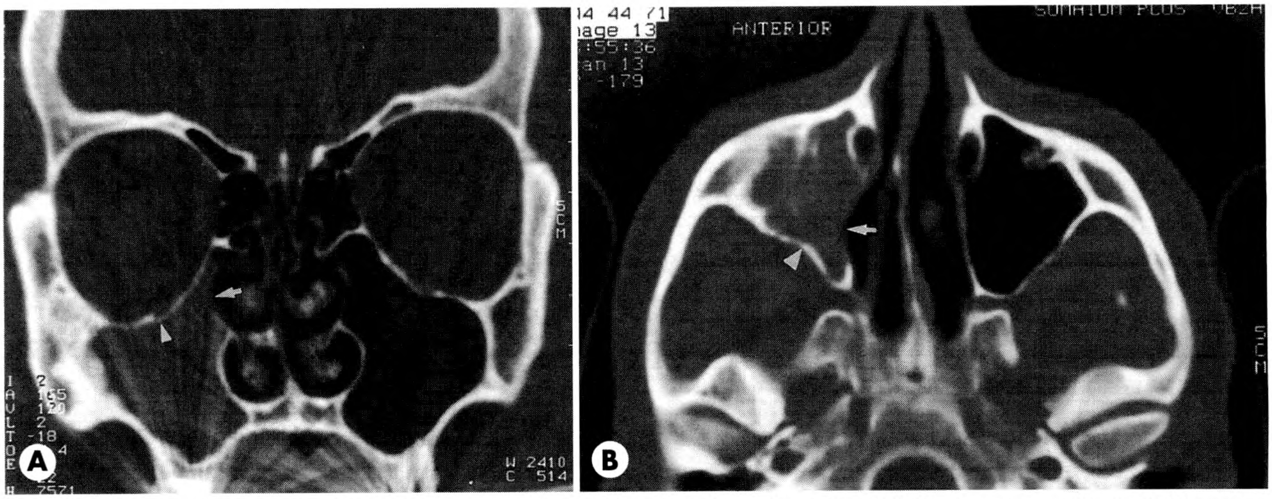

Fig 4. (Case 5) A) Coronal and B) axial computed tomograms of patient with stage II CMA. Arrows — retracted MIW and fontanel, arrowheads — deformed osseous antral walls.

congestion. She had a long history of environmental allergies. There was a septal deviation that limited the intranasal endoscopic examination. She did not have enophthalmos, hypoglobus, or a malar depression deformity. Computed tomography showed complete opacification of the right maxillary sinus, lateral retraction of the fontanel and MIW, sagging of the orbital floor, and inward bowing of the posterolateral wall of the antrum (Figs 4 and 5). The patient was found to have stage II CMA and underwent a septoplasty and MMA. The MIW was a flimsy membranous wall devoid of bone posteriorly. It was laterally positioned and occluded a normal-appearing MSO. The antrum was filled with thick gray secretions and there were no osseous dehiscences of the antral walls; however, a sag to the orbital floor was appreciated. The histopathology showed chronic inflammation with mucus retention and mild stromal eosinophilia. The patient had an unremarkable postoperative course and was doing well at her 1-year follow-up visit.

*Case 9*. An otherwise healthy 45-year-old man presented with diplopia. He also noticed that his right eye appeared to be sinking in. These problems seemed to progress slowly over about a 1-year period. He was otherwise asymptomatic at the time of presentation and denied any history of allergies, facial trauma, or sinus surgery. A nasal examination was unremarkable and there was no malar depression deformity. On ophthalmological examination there was enophthalmos, upper lid retraction, and downward displacement of the right globe. Computed tomography showed complete right antral opacification with inward bowing of the medial, superior, anterior, and posterolateral walls (Figs 6 and 7). The patient received a diagnosis of stage III CMA and underwent a right Caldwell-Luc procedure with a nasoantral window. The antrum was filled with thick gray secretions and the floor of the orbit had a small bony dehiscence lateral to the infraorbital nerve. The posterolateral wall was found to be intact. The orbital floor was repaired via a transconjunctival approach using autologous bone and porous polyethylene sheet allograft (Medpor/Ronex Surgical, College Park, Ga).

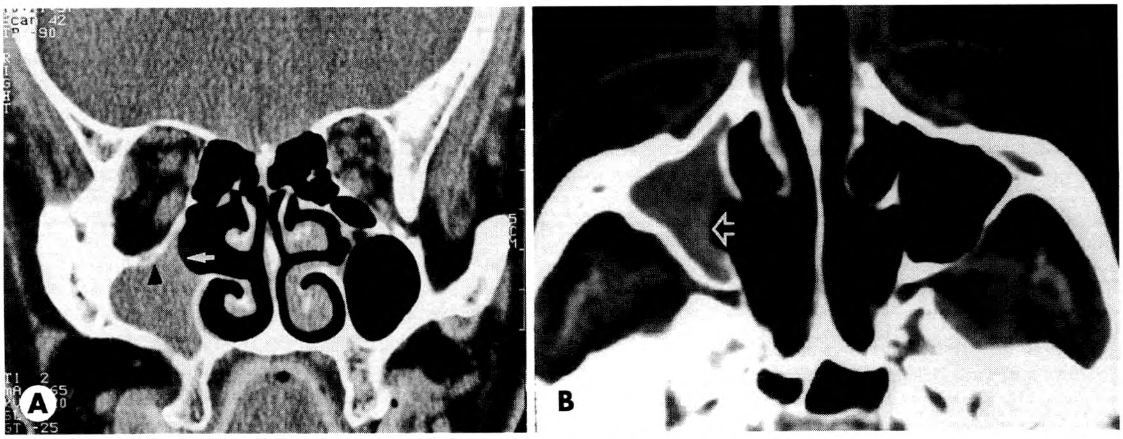

Fig 5. (Case 18) A) Coronal and B) axial computed tomograms of patient with stage II CMA. Arrows — retracted MIW and fontanel, arrowhead — deformed osseous antral wall.

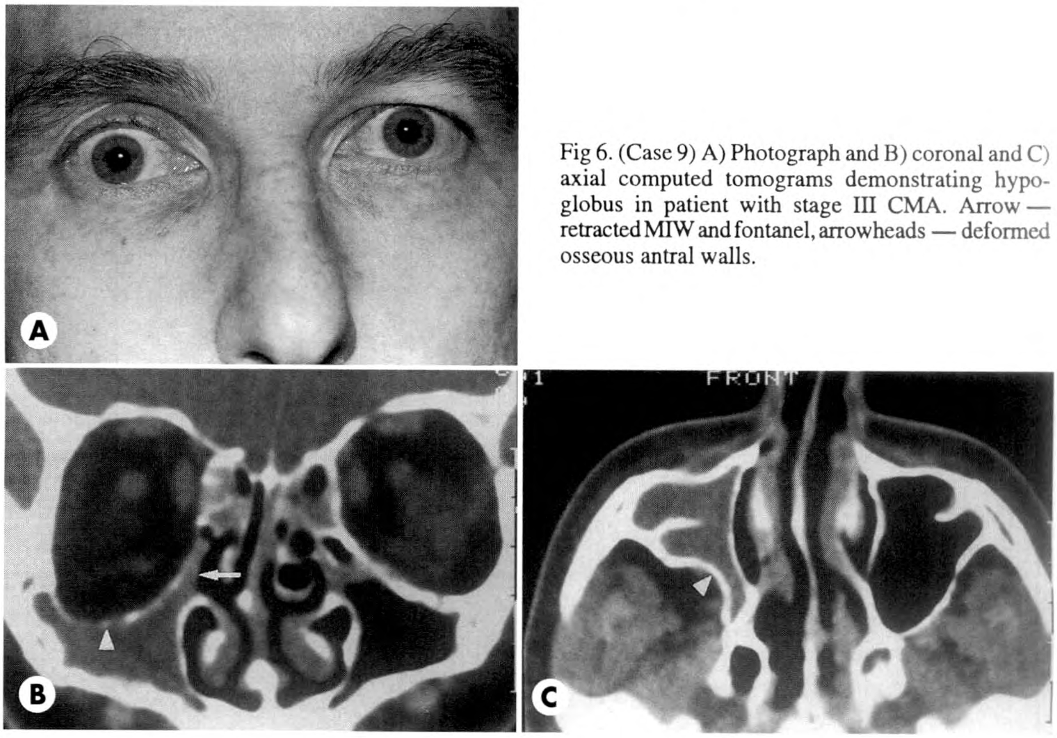

Fig 6. (Case 9) A) Photograph and B) coronal and C) axial computed tomograms demonstrating hypoglobus in patient with stage III CMA. Arrow — retracted MIW and fontanel, arrowheads — deformed osseous antral walls.

The histopathology showed chronic inflammation and mucus retention with mild focal eosinophilia. Microscopic inspection of bone fragments showed moderate osteoclastic and osteoblastic activity consistent with active bone remodeling. The patient was doing well at his 1-year follow-up visit with no visual or sinus-related complaints. The right eye was found to be well positioned and examination of the nose yielded unremarkable findings.

## RESULTS

In this series of 22 patients (Table 3), the average age at the time of diagnosis was 38.3 years, with a standard deviation of 14.5 years. This compared closely with the average age of 37.1 years for the patients from the literature review. There was a

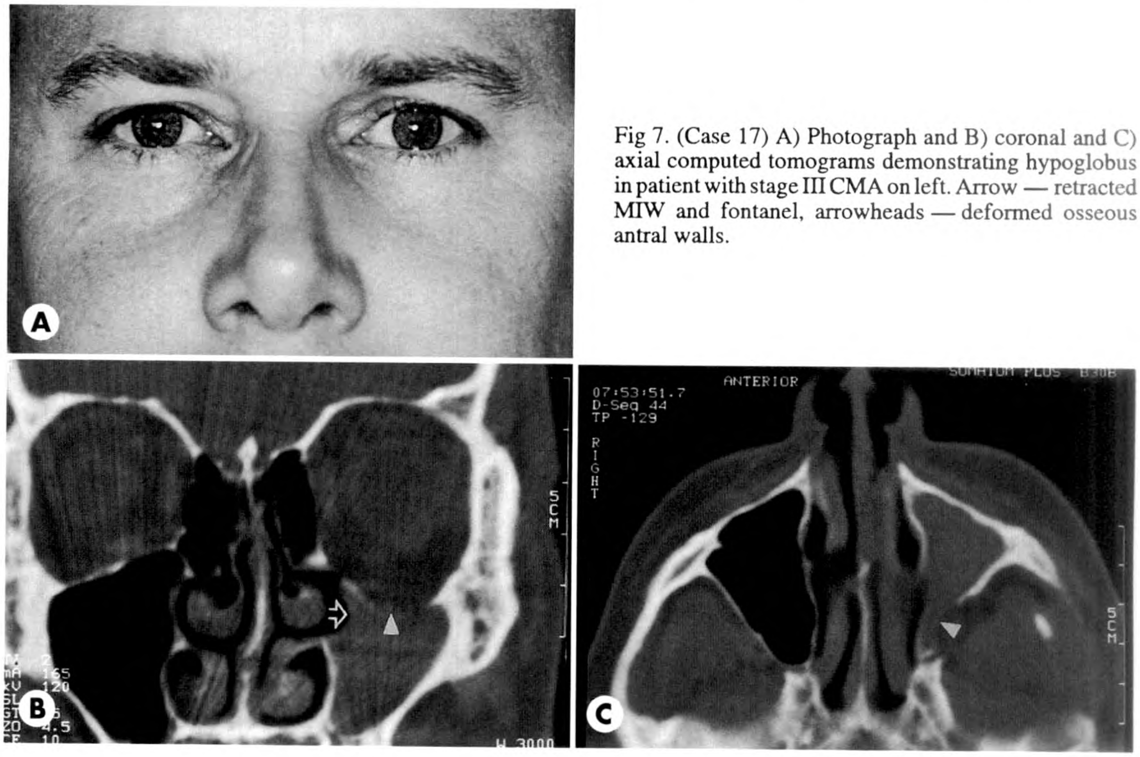

Fig 7. (Case 17) A) Photograph and B) coronal and C) axial computed tomograms demonstrating hypoglobus in patient with stage III CMA on left. Arrow — retracted MIW and fontanel, arrowheads — deformed osseous antral walls.

TABLE 3. SUMMARY OF STUDY CASES OF CHRONIC MAXILLARY ATELECTASIS

| *Case* | *Age* | *Sex* | *Allergies* | *Fontanel Retraction* | *Sinus Opacification* | *Symptoms** | *Side of Involvement* | *Osseous Wall Deformity* | *Hypoglobus/ Enophthalmos* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 38 | F | Y | Y | Y | 1 | L | N | N |
| 2 | 32 | F | Y | Y | Y | 2 | R | N | N |
| 3 | 64 | M | N | Y | Y | 2 | B | N | N |
| 4 | 21 | F | N | Y | Y | 2 | R | N | N |
| 5 | 38 | F | Y | Y | Y | 1 | R | Y | N |
| 6 | 19 | M | N | Y | Y | 0 | R | N | N |
| 7 | 28 | F | Y | Y | Y | 2 | L | N | N |
| 8 | 35 | F | N | Y | Y | 0 | L | Y | Y |
| 9 | 45 | M | N | Y | Y | 0 | R | Y | Y |
| 10 | 35 | M | N | Y | Y | 1 | R | Y | Y |
| 11 | 41 | F | N | Y | Y | 2 | L | N | N |
| 12 | 72 | F | N | Y | Y | 2 | R | N | N |
| 13 | 37 | M | Y | Y | Y | 1 | L | Y | N |
| 14 | 21 | F | Y | Y | Y | 2 | L | N | N |
| 15 | 64 | M | N | Y | Y | 1 | R | N | N |
| 16 | 38 | F | N | Y | Y | 2 | R | N | N |
| 17 | 41 | M | Y | Y | Y | 1 | L | Y | Y |
| 18 | 58 | M | N | Y | Y | 2 | R | Y | N |
| 19 | 30 | F | Y | Y | Y | 2 | B | N | N |
| 20 | 33 | F | N | Y | Y | 2 | L | Y | Y |
| 21 | 27 | F | N | Y | Y | 2 | L | N | N |
| 22 | 26 | F | N | Y | Y | 2 | L | Y | N |

*0 — no recent sinonasal symptoms, 1 — mild sinonasal symptoms (no pain or pus), 2 — moderate to severe sinonasal symptoms (pain and/or pus).

female gender predominance (14:8), and the condition occurred with equal frequency on each side. Symptoms were present in 86.4% of the group, and most (68.4%) of these patients experienced moderate to severe symptoms. Environmental allergies were present in 36.4% of the patients. Symptoms were mild or absent at the time of presentation in 6 of 9 patients with osseous deformity of the sinus walls versus only 3 of 13 without bone deformities ($\chi^2 = 4.180$, $df = 1$, p = .041). The percentage of patients with osseous deformity who lacked significant symptoms in this study compared closely with the 67% found in the literature review. Enophthalmos with hypoglobus occurred in 5 patients; however, midfacial deformities were not seen in our series. Four of the 5 patients with hypoglobus had sinus-related symptoms that were either absent or mild in nature at the time of presentation.

The maxillary sinus was solely diseased in all but 3 of the patients in our series, and an MMA was done in 13 of them. Of the 3 patients who had undergone a Caldwell-Luc procedure and nasoantral window, 1 also had a repair of the orbital floor. Four patients had an endoscopic ethmoidectomy in addition to an MMA. Intraoperative findings included a laterally retracted MIW occluding a normal-appearing MSO, a retracted fontanel, no accessory ostium, and thick gray secretions filling the maxillary sinus. The histopathology consistently showed chronic inflammation with mucus retention. Active bone remodeling was seen in cases in which osseous deformity was present on CT. Patients who underwent surgery were doing well, with no clinical evidence of recurrent disease, at follow-up visits that ranged from 6 months to 5 years postoperatively.

## DISCUSSION

The prevalence of CMA in the general population is unknown, but it is thought to be an uncommon condition.[4,16] In this study, the atelectatic maxillary sinus of several patients was found to be well developed; this finding suggests that the onset of the disorder occurred during late adolescence or adulthood in these instances. For most of the patients in the study the maxillary sinus showed various degrees of hypoplasia, indicating onset during childhood. It is suspected that for many patients the disease may remain in an arrested or subclinical state for months to years. For some patients the condition may undergo slow progression with osseous remodeling and wall deformation. Recent evidence also indicates that there may be shorter periods of accelerated bone loss and wall deformation.[1-3,5-10]

The cause remains elusive some 30 years after its first description.[1] This is probably due in part to the low incidence of the disorder. Several authors believe CMA is best explained as an uncommon sequela of chronic rhinosinusitis[4-6,9]; however, the generally indolent clinical course, unusual structural deformities found in the MIW and/or antral wall, and consistently thick and tenacious mucus found within the antrum argue otherwise. Mucosal inflammation undoubtedly plays a part in the pathophysiology of this disorder, but evidence of an unusual biologic response to inflammation[2] could not be found in this study.

Chronic maxillary atelectasis is considered by some to be a class of maxillary sinus hypoplasia rather than a disease.[11] As mentioned, radiographic evidence in this study showed that many adults with CMA had well-developed maxillary sinuses at the time of presentation. Maxillary sinus hypoplasia did, however, appear to occur more commonly, according to the radiologist's subjective criteria, when compared with the incidence reported in the literature.[11,17] Since diminished sinus volume is a key finding in both conditions, an objective definition of hypoplasia and a more careful analysis are necessary before any firm conclusions can be drawn.

Intraoperative endoscopic study findings repeatedly showed that a laterally positioned MIW was occluding a normal-appearing MSO. As expected, an accessory ostium was not identified, and when pressure was applied to the retracted fontanel, thick gray mucus was expressed from the region of the hiatus semilunaris. This finding was consistent with the forced separation of the opposing infundibular walls by secretions emanating from the maxillary sinus. It is suspected that patients who develop CMA are probably predisposed to the condition as a consequence of increased MIW mobility. The increased mobility is thought to enable it to act as a flap for a one-way valve. The establishment of flap valvular occlusion would require the presence of negative antral pressure, which has not yet been shown to occur in CMA.[18,19]

In the literature, several interpretations of CT and physical examination findings in patients who meet diagnostic criteria for CMA have been reported, including congenital absence of the infundibulum,[11] fusion of the uncinate process with the medial wall of the orbit,[4] and complete absence of the MIW.[11] The findings from this study show that the MIW and infundibulum are present but exist in a collapsed state. Moreover, the presence of a maxillary sinus per se refutes the notion of a congenitally absent infundibulum in patients with CMA, since the maxillary antrum forms subsequent to, and as a lateral extension of, the infundibulum in the third fetal month.[20] The collapsed MIW may give the appearance on CT that the uncinate process is fused to the bony wall of the medial orbit, but mucosal-to-mucosal apposition between these structures was found in every instance in this study.[21,22] In short, it is our belief that these reported findings can be explained by the markedly lateralized position of the MIW that obliterates the infundibular space in patients with this condition.

The percentage of patients with CMA who will go on to develop late-stage sequelae, and what factors portend progression to this stage, remain undetermined. The amount of negative pressure, presence of sinus inflammation, degree of prior antral pneumatization, and duration of the untreated state are thought to play important roles.

Many surgical approaches have been used to treat CMA, including the Caldwell-Luc procedure, endoscopic MMA, and endoscopic ethmoidectomy with MMA.[2-4] Although the Caldwell-Luc approach, with the creation of a generous nasoantral window, may correct the problem, there is increased postoperative morbidity. Endoscopic ethmoidectomy may be unnecessary when disease is limited to the maxillary sinus. Moreover, since endoscopic ethmoidectomy involves a more extensive procedure, it poses a greater risk for inadvertent orbital entry in this setting of altered middle meatal anatomy.[4] For patients with CMA alone, an MMA should relatively safely correct the disorder. Reconstruction of the orbital floor in patients with stage III disease can be performed via a transconjunctival or subciliary approach. The surgeon may choose from alloplastic or autogenous implants to reconstruct the orbital floor. Autogenous grafts include bone and cartilage, while alloplastic implants include materials that are nonporous (ie, silicone, Teflon, Supramid, metallic plates, and mesh), porous (ie, hydroxyapatite and porous polyethylene), and absorbable (ie, Gelfilm and Vicryl).[23] Porous polyethylene sheets offer some advantages in that they are easy to manipulate and become biologically fixed and hence are less likely to extrude or migrate.[24]

## CONCLUSION

Chronic maxillary atelectasis is an uncommon disease that occurs over a wide age range. The most common age group at the time of diagnosis is in the third to fifth decades. Most patients have a history of nasal and sinus-related symptoms at the time of presentation that may vary from mild to severe. The ophthalmological signs and symptoms associated with this disease include diplopia, enophthalmos, and hypoglobus. A past history of absent or mild symptoms referable to the nose and sinuses was encountered more often in those patients with osseous wall deformation. Characteristic CT findings of lateral retraction of the fontanel and MIW, inward bowing of the antral wall(s), and persistent sinus opacification were diagnostic for the disorder. Sinuses with orbital floor deformation on CT had features consistent with normal sinus development, while those that did not were usually hypoplastic. The CMA was easily separated into three stages according to the degree of wall deformation.

Patients with this disorder can be expected to have thick gray mucus filling the antrum, a lateralized MIW, and a retracted maxillary fontanel at the time of surgery. The flap valve theory of MSO occlusion by the MIW was supported by this study's findings and raises suspicion that persistent negative pressure may play an important role in the pathophysiology of this disorder. Mild or absent symptoms at the time of diagnosis should not be considered a negative risk factor for the development of facial deformity, especially if the sinus has features consistent with complete pneumatization. An MMA appears to relatively safely correct the problem, with a minimum of patient morbidity, while ensuring that no other underlying disease exists. The MMA should be done with extra care, since marked retraction of the MIW and the possibility of a sagging orbital floor may increase the likelihood of inadvertent orbital entry. Orbital floor reconstruction for patients with stage III disease can be accomplished effectively via a transconjunctival approach using a combination of bone allograft and porous polyethylene sheets.

ACKNOWLEDGMENTS — We thank Cliff A. Megerian, MD, Max L. Goodman, MD, and Joseph B. Nadol, Jr, MD, for their editorial assistance and Richard Cortese for his photographic and technical assistance. We also thank Robert J. Glynn for his statistical support.

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*Ann Otol Rhinol Laryngol* 106:1997

# SURGICAL MORBIDITY OF NECK DISSECTION AFTER CHEMORADIOTHERAPY IN ADVANCED HEAD AND NECK CANCER

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The use of chemotherapy and irradiation for organ preservation attempts to eliminate the need for extensive surgery in patients with advanced squamous cell carcinoma of the head and neck (SCCHN). We sought to characterize the morbidity of surgery in patients who needed surgery after treatment with induction chemotherapy followed by simultaneous chemotherapy and radiotherapy (chemoradiotherapy). The surgical morbidity within the first 30 postoperative days of 17 patients treated in an organ preservation approach between July 1991 and December 1994 was compared with a control group of patients undergoing similar surgical procedures during the same period. The organ preservation study patients underwent surgical procedures consisting of 18 neck dissections and 5 resections of the primary site. Six patients in the organ preservation study group experienced 8 surgical complications within the first 30 postoperative days, and most complications were minor. There was no significant difference in the duration of surgery or length of hospitalization between study patients and matched controls. Our surgical complication rate (35.3%) was higher but not statistically different from that of the control group, and compared favorably to reports of surgical morbidity (44% to 61%) in the literature on patients treated with chemoradiotherapy. The lower complication rate seen in this study may be a reflection of early surgical intervention as part of our organ preservation study scheme, the preponderance of neck dissections performed, and the limited number of pharyngeal procedures performed.

KEY WORDS — morbidity, organ preservation, squamous cell carcinoma, surgery.

## INTRODUCTION

Clinical studies that use induction chemotherapy and chemoradiotherapy for organ preservation in squamous cell carcinoma of the head and neck (SCCHN) have as their primary goal the elimination of the need for extensive surgery in hopes of maintaining normal speech and deglutition. Organ preservation has particular importance for patients with stage III or IV SCCHN, because 5-year survival rates are less than 40%.[1] Organ preservation approaches have not yet shown improved survival for patients so treated; therefore, the evaluation of the safety and complications of this approach is essential.

This paper specifically examines the surgical morbidity of patients enrolled in an organ preservation study who required surgical treatment, either at the primary site or for regional (neck) disease. The surgical morbidity of the organ preservation study group patients is compared to the surgical morbidity of a matched control group of patients, as well as reports in the literature of surgical patients treated with similar strategies for organ preservation.

## PATIENTS AND METHODS

A multidisciplinary organ preservation study that uses induction chemotherapy followed by simultaneous chemoradiotherapy was activated at the Stanford University Medical Center and the Department of Veterans Affairs Medical Center at Palo Alto in July 1991. Patients with resectable stage III or IV SCCHN were eligible for participation in the organ preservation study. This paper focuses only on those patients who received surgery.

The treatment protocol for organ preservation required two cycles of induction chemotherapy using cisplatin and fluorouracil followed by the simultaneous administration of two cycles of cisplatin and fluorouracil during standard fractionated radiotherapy (see Figure and Pinto et al[2]). Disease status and response to treatment were evaluated weekly during the treatment course. Patients achieving a complete response at the end of 50 Gy of radiotherapy received completion radiotherapy to a dose of 66 to 75 Gy and adjuvant chemotherapy: two cycles of cisplatin and fluorouracil. Surgery was recommended for patients who had 1) progressive disease at any time, 2) no response to induction chemotherapy, or 3) less than a complete response after chemoradiotherapy. The evaluation for response to chemoradiotherapy was made when the patient had completed 50 Gy of treatment,

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Presented at the Western Section Meeting of the American Laryngological, Rhinological and Otological Society, Inc, Scottsdale, Arizona, January 13-15, 1995.

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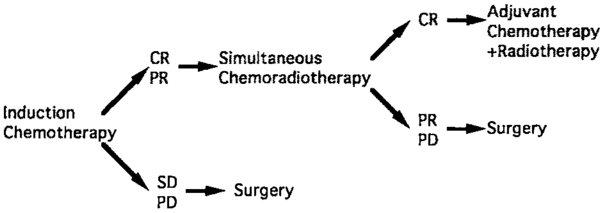
Induction Chemotherapy
CR
PR
Simultaneous Chemoradiotherapy
CR
Adjuvant Chemotherapy +Radiotherapy
PR
PD
Surgery
SD
PD
Surgery

Schema of organ preservation protocol. Induction chemotherapy consists of two cycles of cisplatin and 5-fluorouracil. Chemoradiotherapy consists of administration of radiotherapy in standard fractionated doses and simultaneous administration of two cycles of cisplatin and 5-fluorouracil. Patients are evaluated after radiation dose of 50 Gy, and those with complete response (CR) continue receiving radiotherapy to total dose of 66 to 75 Gy followed by two cycles of adjuvant chemotherapy (cisplatin and 5-fluorouracil). Surgery is undertaken when there is no response to induction chemotherapy, progressive disease (PD), or incomplete response after 50 Gy of radiation. PR — partial response, SD — stable disease.

and it was at this point that surgery was performed for persistent disease either at the primary site or in the neck. The status of the primary site and that of the neck were assessed independently. Patients underwent surgery of the primary site alone if the neck was determined to be without clinical evidence of disease. Surgery was performed in all patients within 2 to 4 weeks of irradiation, except when surgery was performed for recurrent disease.

The surgical procedure performed was determined by the site of the persistent or recurrent disease. In patients who had persistent disease at the primary site, the type of surgery outlined at a pretreatment multidisciplinary evaluation was performed. In patients who had a complete response at the primary site but who also had persistent abnormalities in the neck, a neck dissection was performed. For patients who responded completely but later developed recurrent disease at the primary disease site or regionally, the appropriate salvage surgical procedure was determined at the time of recurrence. All of the surgery was performed by residents under faculty supervision at the Stanford University Hospital and the Palo Alto Veterans Administration Hospital.

The control group included 17 patients treated between December 1991 and December 1994 and matched with the treatment group for factors known to influence the surgical morbidity: preoperative TNM stage, surgical procedure performed, age, and preexisting medical conditions. We evaluated the 30-day surgical morbidity in the two patient groups, with an emphasis on the number and type of perioperative complications, duration of surgery, and length of hospital stay. Student's *t*-test and $\chi^2$ values were used to compare the morbidity experienced between the two patient groups.

Surgical morbidity was defined as any perioperative event that required further surgical intervention or prolonged the patient's hospitalization. These perioperative events were classified on the basis of the level of intervention required for management. Major complications required operative intervention or intensive care unit monitoring, and minor complications were managed with nonoperative intervention and outpatient evaluations.

## RESULTS

Twenty-eight patients enrolled in this organ preservation study between July 1991 and December 1994 and 17 patients underwent surgery during this period. The characteristics of patients who had surgery are listed in Table 1. Thirteen patients had surgery as part of the protocol treatment (they failed to achieve a clinical complete response), 3 patients had surgery for recurrent disease, and 1 patient had surgery to repair a nonhealing glossopharyngeal ulcer that developed after successful treatment of the primary site.

A radical or modified radical neck dissection was performed in 12 patients for regional disease only (2 patients had bilateral neck dissections). In 5 patients, surgery was performed at the primary site, and in 4 of these cases, a neck dissection was also performed. The specific surgical procedure and the surgical complications are shown in Table 1. Six of the 17 patients who had surgery in the experimental group had surgical complications (35.3%).

There were 3 major complications and 5 minor complications in the organ preservation surgical patients. Of the 2 patients that had major complications (11.8%), 1 patient developed 2 major complications: a pharyngocutaneous fistula and a carotid artery rupture. The carotid artery was sacrificed with no permanent neurologic sequelae, and the pharyngocutaneous fistula was repaired with a myocutaneous flap. Another patient had a persistent tracheocutaneous fistula after decannulation and required a surgical procedure to close the defect. There were no postoperative deaths. Four patients developed 5 minor complications (23.5%), including a unilateral chyle fistula in 1 patient, bilateral chyle fistulae in 1 patient, and a seroma in 2 additional patients. These minor complications were treated with aspiration followed by pressure dressings.

A comparison between the surgical patients in the experimental organ preservation group (17 patients) and the control group (17 patients) is shown in Table 2. Two minor complications occurred in the control group (11.8%) — 1 partial flap necrosis and 1 wound

TABLE 1. SURGICAL PATIENTS

| *Patient* | *Gender* | *Age* | *Tumor Site* | *Stage* | *Radiotherapy (Gy)* | | *Surgical Indication* | *Type of Surgery* | *Complications* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Primary* | *Neck* | | | |
| 1 | M | 49 | Base of tongue | T4N2c | 75 | 50 | Persistent neck disease | R MRND,<br>L MRND, Trach* | Left neck seroma |
| 2 | M | 49 | Pyriform sinus | T2N2b | 66 | 50 | Persistent neck disease | R MRND | None |
| 3 | F | 65 | Soft palate | T2N1 | 66 | 50 | Recurrent disease | Partial palatectomy | None |
| 4 | M | 62 | Supraglottis | T4N1 | 66 | 50 | Persistent primary disease | Laryngopharyngectomy, BND, PMF | Fistula, carotid rupture |
| 5 | F | 59 | Base of tongue | T2N2c | 75 | 66 | Persistent neck disease | L MRND, Trach* | None |
| 6 | M | 67 | Lateral pharynx | T2N2b | 66 | 50 | Recurrent disease | L RND | None |
| 7 | M | 58 | Vocal cord | T3N1 | 66 | 50 | Persistent neck disease | L MRND | None |
| 8 | M | 52 | Base of tongue | T4N2b | 72 | 50 | Persistent neck disease | L MRND, Trach* | Persistent tracheocutaneous fistula |
| 9 | M | 47 | Base of tongue | T4N2c | 72 | 60 | Persistent neck disease | R RND | Right neck seroma |
| 10 | M | 64 | Base of tongue | T4N1 | 70 | 50 | Radiation ulcer | Partial glossectomy, PMF | None |
| 11 | M | 46 | Base of tongue | T4N2c | 74 | 50 | Persistent neck disease | L MRND | Chyle leak |
| 12 | M | 46 | Base of tongue | T3N0 | 66 | 50 | Recurrent disease | Partial glossectomy, L MRND | None |
| 13 | M | 56 | Base of tongue | T4N3 | 66 | 50 | Persistent neck disease | L RND | None |
| 14 | M | 46 | Supraglottis | T3N3 | 66 | 50 | Persistent neck disease | R RND | None |
| 15 | M | 58 | Base of tongue | T4N2c | 50 | 50 | Persistent primary disease | Partial glossectomy, BND | None |
| 16 | M | 60 | Base of tongue | T4N3 | 74 | 50 | Persistent neck disease | R RND,<br>L MRND | Right and left neck chyle leak |
| 17 | M | 43 | Oral tongue | T4N1 | 48 | 46 | Persistent neck disease | L MRND | None |

MRND — modified radical neck dissection, Trach — tracheotomy, BND — bilateral neck dissection, PMF — pectoralis myocutaneous flap, RND — radical neck dissection.

*Cases in which interstitial radiotherapy implant catheters were placed into primary site at time of neck dissection.

infection — and this was not statistically different from the organ preservation group (p = .65). There were no major complications in the control group (0.0%), and this proportion did not differ significantly from the organ preservation group (p = .47). Eleven patients in each surgical group had similar neck dissections performed as isolated surgical procedures whereby quantitative measures of operating time and duration of hospitalization could be performed. The average duration of surgery (± standard deviation) for a neck dissection was 4.1 ± 0.3 hours in the organ preservation group and was 4.1 ± 0.4 hours in the control group (p = .89). The duration of surgery was obtained from the anesthetic record, which initiated the start time during patient draping and concluded when the drapes were removed by the surgical team. Length of hospital stay for a neck dissection was 5.0 ± 1.7 days in the organ preservation group and 4.1 ± 1.3 days in the control group (p = .19).

## DISCUSSION

The purpose of this study was to evaluate the surgical morbidity in patients treated with induction chemotherapy and chemoradiotherapy and to compare it to that in patients who did not have preoperative treatment. Although we found our complication rate was higher when compared to a matched control group, our statistical analysis is limited, because the number of patients in each group is too small to reliably discern differences between the groups. Our organ preservation surgical morbidity results com-

TABLE 2. COMPARATIVE SURGICAL DATA

| *Characteristic* | *Experimental Group (n = 17)* | *Control Group (n = 17)* |
|---|---|---|
| Age (y; mean ± SD) | 54.4 ± 7.8 | 55.1 ± 9.9 |
| Operating room time for neck dissections (h; mean time per neck; n = 11) | 4.1 ± 0.3 | 4.1 ± 0.4 |
| Hospitalization for neck dissections (d; mean time per admission; n = 11) | 5.0 ± 1.7 | 4.1 ± 1.3 |
| Minor complication rate (%; patients with complications/ total surgical patients) | 23.5 | 11.8 |
| Major complication rate (%; patients with complications/ total surgical patients) | 11.8 | 0.0 |

pare favorably with reports of surgical morbidity in patients receiving preoperative radiotherapy, induction chemotherapy, or chemoradiotherapy.

The incidence of complications in patients treated with preoperative radiotherapy has been reported, and there is general agreement that preoperative radiotherapy for advanced SCCHN increases the risk for pharyngocutaneous fistulae, wound complications, and carotid rupture.[3-5] Complication rates of 40%,[6] 43%,[3] and 54%[7] have been reported in patients treated with 50 Gy prior to surgery. When higher doses of preoperative radiotherapy (50 to 70 Gy) are administered, the rate of pharyngocutaneous fistula alone is approximately 31%[4] to 34%.[3]

There have been few systematic studies of the surgical morbidity of patients treated with induction chemotherapy. Early chemotherapy studies using methotrexate prior to surgery reported significantly greater surgical complications in comparison to randomized patients receiving no preoperative chemotherapy.[8,9] More recent studies using induction-based cisplatin chemotherapy have shown no greater proportion of surgical complications when compared to no chemotherapy.[10-13] Lore et al[13] found that 19% of 42 patients developed surgical complications after induction chemotherapy with cisplatin, vincristine, and bleomycin; there were no carotid ruptures or postoperative deaths, but 8% developed wound infections and 11% developed a fistula. Schuller et al[14] found that 46% of 61 patients developed surgical complications after induction with cisplatin, methotrexate, and bleomycin. Similarly, Davidson et al[15] found that 48% of 88 patients treated with initial radiotherapy with or without a split course of mitomycin and fluorouracil developed surgical complications.

Higher surgical morbidity has been reported in patients undergoing organ preservation that utilized three cycles of induction chemotherapy of platinum and fluorouracil followed by definitive radiotherapy.[16] That study reported a 61% incidence of major wound complications and 2 deaths in 18 patients who underwent surgical salvage for recurrent disease. Those patients differed from our organ preservation study patients because they completed their definitive radiotherapy course with partial responses at 50 Gy. In contrast, patients in that category would have undergone surgery at that point in our organ preservation scheme. Other authors[17] have noted higher surgical wound complication rates in patients treated with full-course chemoradiotherapy despite evidence of persistent or progressive disease during their treatment.

The type and proportion of surgical complications in our series are similar to those reported in other studies.[3-9,13-16] One exception was the incidence of chyle fistulae (11.8%), which was higher than the usual incidence of 1% to 3% reported in the literature.[18] Two fistulae in our series were recognized intraoperatively, and attempted repair with suture and fascia oversewing was unsuccessful. All chyle fistulae in our series resolved after aspiration and pressure dressings in our outpatient clinics. We are unable to identify any known factor in this small number of patients that could explain this higher proportion of chyle fistulae. The most serious complication (carotid artery rupture) occurred within the small subset of patients undergoing resection of their primary site. There were no major complications attributed to neck dissections in this study. The complication of wound infection was noticeably absent from the organ preservation study patients. Patients in this study received preoperative doses of cefazolin and metronidazole, which were maintained postoperatively for 24 hours in the majority of cases.

The most common surgical procedure performed in our study was neck dissection. The duration of surgery for the neck dissections in this study reflects the fact that all operations were performed by residents, usually in their second or third year of training, with teaching by staff physicians. With the exception of 3 cases, the neck dissections were performed through MacFee incisions and closures performed with three-layer suture reapproximation. The duration of hospitalization was most closely related to the period of time in which patients required active surgical drains. The complications of seroma and chyle fistula were detected at postoperative clinic visits and did not impact upon the duration of hospitalization.

After the completion of chemoradiotherapy, patients in our study were treated with surgery if they had any clinical evidence of residual disease. We treated the neck with surgery if any clinical and/or radiologic evidence of persistent disease existed at the completion of 50 Gy. We favor early surgery as an appropriate strategy because induction chemotherapy has only improved survival in patients with unresectable disease,[19] and organ preservation remains experimental, as its impact on survival is currently being assessed. Furthermore, Wolf and Fisher[20] suggest that early neck dissection may have an impact on survival for patients treated with an organ preservation approach.

The proportion of complications was higher in our organ preservation surgical patients than in a group of matched control patients, yet lower than in patients completing definitive chemoradiotherapy in other, larger studies.[14-16] Two important points contributed to this finding. In our study, surgical intervention was performed for both partial responders and nonresponders after 50 Gy treatment, in contrast to having partial responders complete full-course irradiation prior to surgical intervention. These latter patients who experienced higher complication rates in other studies received doses of radiotherapy in excess of 65 Gy prior to surgical intervention. Second, 78% of surgical cases performed in our series (18 of 23) were neck dissections, with only 5 cases involving pharyngeal procedures; thus, the potential for fistula was reduced. The surgical morbidity rate in our study most closely reflects that of patients undergoing neck dissection. The limited number of patients undergoing pharyngeal procedures precludes us from drawing conclusions about comparative morbidity in this subset of patients. The majority of surgical complications encountered in this study were minor and were managed satisfactorily with conservative outpatient treatment.

We believe that the findings of surgical morbidity in this study suggest that neck dissection may be performed with acceptable morbidity in patients undergoing chemoradiotherapy as part of organ preservation. Serious complications may occur in patients requiring pharyngeal procedures. Larger studies of surgical morbidity involving the primary site are needed in order to properly address the balance between attempting to achieve organ preservation and preserving the life of the patient.

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## TWENTY-FOURTH NES CONGRESS

The Twenty-Fourth Ordinary Congress of the International Neurootological and Equilibriometric Society Reg. (NES) will be held April 6-10, 1997, in Haifa, Israel. For information, contact NES Congress President Prof Dr Ludwig Podoshin, Otolaryngology, Head and Neck Surgery, Bnai Zion Medical Center, PO Box 4940, Haifa 31048, Israel; telephone 972-4-359544; fax 972-4-371393.

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## INNER EAR MEDICINE AND SURGERY

The Sixth Symposium and Workshops on Inner Ear Medicine and Surgery, combined with the Prosper Meniere Society Annual Winter Meeting, will be held in Aspen/Snowmass, Colorado, March 22-29, 1997. For further information, contact Prosper Meniere Society, c/o Jane Wells, Meeting Coordinator, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

*Ann Otol Rhinol Laryngol* 106:1997

# USHER'S SYNDROME TYPE IC: CLINICAL STUDIES AND FINE-MAPPING THE DISEASE LOCUS

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Usher's syndrome type I is a heterogeneous group of diseases characterized by severe to profound sensorineural hearing loss, absent vestibular function, and progressive pigmentary retinopathy. Other identifying clinical features have not been documented. In this study, we examined olfactory acuity, plasma levels of polyunsaturated fatty acids and sarcosine, and cilia ultrastructure in a homogeneous cohort of patients with Usher's syndrome type IC. The normal age-dependent decline in olfactory acuity was observed, and normal plasma levels of polyunsaturated fatty acids and sarcosine were found. However, the incidence of compound cilia in biopsies from the inferior turbinate was significantly higher than that reported in control populations. By reconstructing haplotypes in affected persons, D11S902 and D11S1310 were identified as flanking markers over an interval that contains a candidate gene, KCNC1. No mutations in the coding sequence of this gene could be demonstrated in affected persons.

KEY WORDS — compound cilia, linkage disequilibrium, Usher's syndrome type IC.

## INTRODUCTION

The Usher's syndromes (USHs) are characterized by autosomal recessive inheritance and dual sensory impairments. Affected persons have congenital sensorineural hearing loss (SNHL) and suffer gradual visual impairment secondary to progressive pigmentary retinopathy (PPR). Progressive pigmentary retinopathy is the sine qua non for the diagnosis of this disease, with classification into distinct types of USH based on clinical tests of auditory and vestibular function.[1] These tests do not differentiate between subtypes of USH, and if this type of distinction is to be made, genotyping is required[2-7] (Table 1). Identification of additional phenotypic abnormalities in USH could potentially obviate this requirement. In the case of some USH subtypes, efforts to identify USH genes also would be facilitated.

Proteins associated with structural elements found in both the eye and ear are obvious candidate genes for USH. Several studies have analyzed ultrastructural features of the cilium, an extension of the cell surface common to both sensory systems. Retinal sensory cilia connect outer and inner membrane discs and serve a transport function. Ultrastructurally, they have nine outer microtubule doublets that originate from a basal body but lack central paired singlet microtubules, radial spokes, and dynein arms. In a heterogeneous group of USH patients (9 USH2 patients, subtype unknown; 1 USH1 patient, subtype unknown), Hunter et al[8] documented abnormalities of the outer doublets in 60% of receptor axonemes. Barrong et al[9] studied a single USH2 patient (subtype unknown) and also found abnormalities in microtubule number in 86% of cilia; no cilia abnormalities were found in patients with retinitis pigmentosa (RP).

Stereocilia in the inner ear mediate conduction in both the vestibular and auditory systems. Although true sensory cilia are absent, each vestibular hair cell

TABLE 1. USHER'S SYNDROME TYPES AND LOCI

| *Usher's Syndrome* | *Chromosomal Assignment* |
|---|---|
| Usher's syndrome type I | |
| Type IA (USH1A) | 14q32 (2) |
| Type IB (USH1B) | 11q13.5 (1, 3) |
| Type IC (USH1C) | 11p15 (1) |
| Type ID (USH1D) | Unknown |
| Usher's syndrome type II | |
| Type IIA (USH2A) | 1q42 (4, 5) |
| Type IIB (USH2B) | Unknown |
| Usher's syndrome type III | |
| Type III (USH3) | 3q21-25 (6) |

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TABLE 2. SCREENING PRIMERS FOR KCNC1 MUTATIONS

| *Primer Pair* | *Sequence* | *Product Size (bp)* |
|---|---|---|
| 1 | ATGGGCCAAGGGGACGAGAGC<br>ATGTGCGCGAAGACGCCGGGGT | 200 |
| 2 | GCACATCCTGAACTACTACCG<br>GCTGTTGTCCAGAGGAGCGCC | 194 |
| 3 | GACAACAGCGCCGACGACG<br>CGCGTAGCGGGACGAGTA | 186 |
| 4 | ATGTGGCCTTCGCTTCCCTC<br>ACCTCCAGGTAGAAGGGCAGG | 306 |
| 5 | TTCTACCTGGAGGTGGGGCTG<br>GCGTAGTAGATCATGGTGGC | 233 |
| 6 | TACGCCGAGAGGATAGGGGC<br>CACGATGACGGGCACGGGCA | 215 |
| 7 | CATCGTGAACAATTTCGG<br>GATCGACATGCCTCTGAGAG | 231 |

Intronless gene, overlapping primers.

has a kinocilium, and during embryogenesis, one also is present on each cochlear hair cell.[10] Distinct from motile and sensory cilia, inner ear kinocilia lack dynein arms, but do have nine outer microtubule doublets and a pair of central singlet microtubules. Four temporal bones have been harvested from persons with presumed USH[11-13]; however, from the historical information provided, it is unlikely that one of these individuals had USH.[11] The remaining cases probably represent two examples of USH1 (subtype unknown)[12] and one example of USH3.[13] In none is an ultrastructural examination of kinocilia reported.

Although the olfactory epithelium represents a third type of ciliated sensory epithelium, its ultrastructure has not been studied in USH patients, primarily because of anatomic inaccessibility.[14] However, nasal cilia have been studied in 6 persons with USH (type unspecified), and a higher than normal incidence of abnormal axonemal structures and compound cilia have been reported.[15,16] Studies of semen structure and function in persons with USH (10 USH males: 9 with USH2, subtype unknown, 1 with USH1, subtype unknown) also have demonstrated significant axonemal abnormalities at the light and electron microscopic levels and decreased sperm motility and velocity.[8]

In addition to studies of cilia, because the central nervous system is rich in membrane phospholipids derived from esterified polyunsaturated fatty acids (PUFAs) that are essential for normal neurological function, serum levels of docosahexaenoic acid (22:6 ω3) and arachnidonic acid (20:4 ω6) have been quantitated in USH patients. Both Bazan et al[17] (number of patients not given, USH type not specified) and Williams et al[18] (7 patients, USH type not specified) found a significant decrease in these two PUFAs. One abnormality of amino acid metabolism, sarcosinemia, also has been reported in association with USH[19]; however, any relationship is tenuous, as other studies have found it difficult to ascribe a particular disease phenotype to this defect.[20]

TABLE 3. HAPLOTYPES OF 18 PERSONS WITH USHER'S SYNDROME TYPE 1C

| *D11S419* | *D11S902* | *D11S921* | *D11S1310* | *No. of Chromosomes* |
|---|---|---|---|---|
| 2 | 4 | 3 | 1 | 28 |
| 2 | 7 | 3 | 2 | 1 |
| 2 | 4 | 3 | 3 | 3 |
| 2 | 4 | 3 | 2 | 2 |
| 3 | 7 | 3 | 2 | 1 |
| 3 | 7 | 3 | 3 | 1 |

Alleles arbitrarily numbered; size of common D11S921 allele, 247 base pairs.

The purpose of this study was to evaluate olfactory acuity, nasal cilia ultrastructure, and plasma levels of PUFA and sarcosine in a homogeneous cohort of USH1C patients. To ensure homogeneity, all individuals with the clinical diagnosis of USH1 were haplotyped by means of microsatellite repeat polymorphic markers linked to the USH1C locus.[4] This information refined localization of the USH1C gene and identified a candidate gene.

## MATERIALS AND METHODS

### PATIENT IDENTIFICATION

Individuals with the presumed diagnosis of USH1 were examined at the Catholic Deaf Center, Lafayette, La. The diagnosis of USH1 was established by clinical criteria.[1]

### GENOTYPING

To determine individual genotypes, 30 mL of blood was collected from each person in ethylenediaminetetraacetic acid (EDTA)–containing tubes. Genomic DNA was prepared from peripheral lymphocytes[21] and amplified by the polymerase chain reaction (PCR) as previously described.[4] Markers selected for analysis were known to be tightly linked to the USH1C locus.[4]

### SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS

Oligonucleotide primers for single-strand conformation polymorphism (SSCP) were identified by analyzing the coding sequence of KCNC1[22] with Primer 0.5 and synthesized by standard phosphoramidite chemistry on an Applied Biosystems model 391 DNA synthesizer (Table 2). Labeled PCR products for SSCP analysis were obtained by combining

TABLE 4. OLFACTORY ACUITY IN PERSONS WITH USHER'S SYNDROME TYPE 1C

| | *Normosmia* | | *Microsomia* | | *Anosmia* | |
|---|---|---|---|---|---|---|
| *Age Group* | *Affected* | *Carrier* | *Affected* | *Carrier* | *Affected* | *Carrier* |
| 6-10 | | 1 | | | | |
| 11-15 | | | | | | |
| 16-20 | 1 | | | | | |
| 21-25 | | 1 | | | | |
| 26-30 | 1 | 1 | | | | |
| 31-35 | | | | | | |
| 36-40 | 1 | | 1 | | | |
| 41-45 | 1 | | 3 | | | |
| 46-50 | | | | | | |
| 51-55 | 2 | | 1 | | | |
| 56-60 | | 1 | 1 | | | |
| 61-65 | | | 3 | 1 | | |
| 66-70 | 1 | | | | | |
| 71-75 | | | 1 | | | |

Normosmia, microsmia, and anosmia as defined by Doty.[23]

1 µL10× PCR buffer; 100 ng genomic DNA; 50 pmol of each primer; 200 nmol each of dCTP, dGTP, and dTTP; 2 nmol cold dATP; 0.4 µL [35-S]dATP (1,000 Ci/nmol, 10 µCi/mL); and 0.1 unit of Taq DNA polymerase (total volume 10 µL). Each reaction was overlaid with mineral oil, and amplification was carried out for 30 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Labeled amplified DNA was mixed with an equal volume of formamide loading dye (95% formamide; 20 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). Samples were denatured for 5 minutes at 95°C, loaded onto a 6% acrylamide gel (6% acrylamide [49:1 acrylamide:bis], 5% vol/vol glycerol, and 0.5% TBE), and electrophoresed for at least 2.5 hours at 20 W (0.5 W/cm) at room temperature in 0.5× TBE running buffer. Constant temperature was maintained with a cooling fan. Genomic DNA from affected and nonaffected persons, including known carriers, was studied.

PHENOTYPIC STUDIES

*Olfactory Acuity.* Olfactory acuity was measured with the Smell Identification Test (Sensonics, Inc). This forced-choice test measures the ability to recognize 40 odorants. Normative data have been well established,[23] and the test is simple to administer. An American Sign Language interpreter assisted each affected person with the test. No time limit was imposed.

TABLE 5. INCIDENCE OF COMPOUND CILIA IN PERSONS WITH USHER'S SYNDROME TYPE 1C

| *Patient* | *Compound Cilia* | *Total Cilia* |
|---|---|---|
| 4 | 3 | 50 |
| 11 | 2 | 47 |
| 12 | 4 | 124 |
| 15 | 2 | 51 |

Quantitated by electron microscopy.

*PUFA and Sarcosine.* Blood samples were obtained to determine plasma levels of PUFA and sarcosine. Samples were analyzed by chromatography at The Kennedy Institute, Baltimore, Md.

*Cilia Ultrastructure.* Nasal mucosal biopsies were taken from the right inferior turbinate using topical anesthesia, endoscopic visualization, and micro-instrumentation. Biopsy specimens were placed in glutaraldehyde, and prepared and mounted in Spurr's resin. After sectioning and staining with uranyl acetate and lead citrate, individual sections were examined at the Central Electron Microscopy Research Facility under a Hitachi H-600 transmission electron microscope.

## RESULTS

GENOTYPING

Twenty-nine persons of Acadian extraction were haplotyped with markers D11S419, D11S902, D11S921, and D11S1310.[24] Of the 18 persons with USH1C, 13 were homozygous at all four markers, 4 were homozygous for three markers (D11S419, D11S902, D11S921), and 1 was homozygous for two markers (D11S921, D11S1310). All18 shared the same allele at D11S921. Of the 7 known carriers and 4 unrelated controls, none was homozygous at D11S921 (Table 3).

SINGLE-STRAND CONFORMATION POLYMORPHISM ANALYSIS

No mutations were detected in the coding sequence of KCNC1 by SSCP analysis of 5 affected

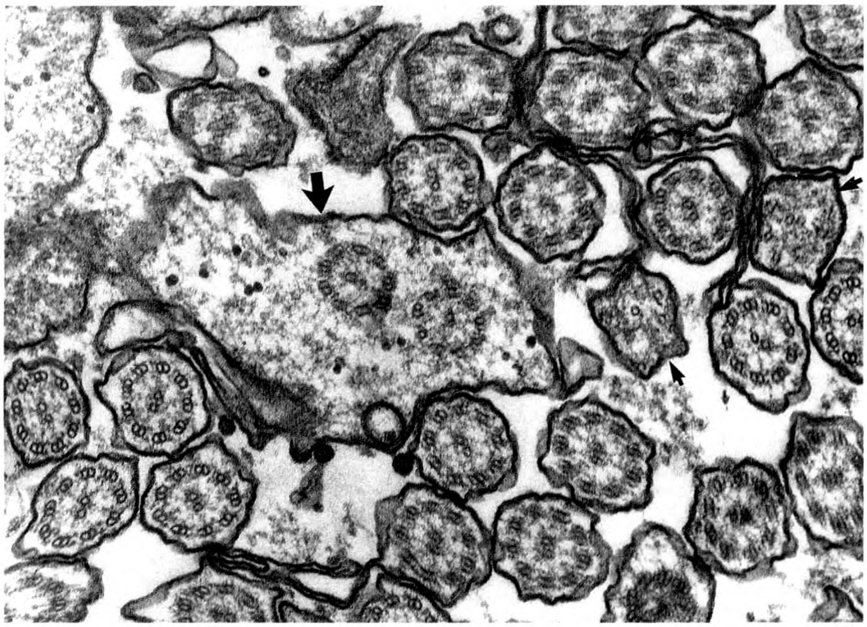

Fig 1. Representative example of nasal mucosal biopsy showing compound cilia (large arrow) and cilia with ultrastructural abnormalities (small arrows); ultrastructural abnormalities can be extremely difficult to recognize and were not quantitated (original ×76,000).

persons and 5 known carriers compared with 5 controls.

PHENOTYPIC STUDIES

*Olfactory Acuity*. The age-related decrease in olfactory acuity in persons with USH1C was not significantly different from that observed in a control population ($p = .15$; Table 4[23]).

*PUFA and Sarcosine*. Plasma levels of docosahexaenoic acid (22:6 ω3), arachnidonic acid (20:4 ω6), and sarcosine were within two standard deviations of normal for all persons with USH1C ($n = 18$).

*Cilia Ultrastructure*. The internal structure of the axoneme of a cilium has a 9 + 2 microtubular pattern, although variations in this pattern occur in sections taken from the tip or base.[15] Cilia were identified in 9 of 29 specimens; however, in 3 specimens, fewer than 10 cilia could be counted. In each of the remaining specimens (4 from persons with USH1C), a minimum of 25 cilia were identified (average, 60). In the persons with USH1C, the percentage of compound cilia (cilia containing more than one 9 + 2 microtubular arrangement within a single axoneme) ranged from 3.2% to 6% (average, 3.9%; Table 5). Microtubular abnormalities defined as failure to conform to the classic 9 + 2 arrangement or to the structure characteristic of the base or tip are notoriously difficult to classify and were not quantitated (Fig 1).

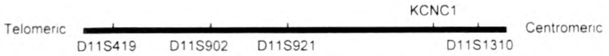

Fig 2. Region of Usher's syndrome type 1C gene showing polymorphic markers (below line) and relative position of KCNC1. Flanking markers D11S902 and D11S1310 are approximately 800 kB apart.

DISCUSSION

To ensure population homogeneity, all individuals with the clinical diagnosis of USH1 were haplotyped by means of microsatellite repeat polymorphic markers linked to the USH1C locus.[4] Results were in agreement with data reported by Keats et al[25] and demonstrated that the USH1C gene is in linkage disequilibrium with flanking markers. Haplotyping ensured that clinical tests were performed on a homozygous USH1C population and further refined localization of the USH1C gene from the 2-3 cM D11-S902-D11S899 interval[25] to the 0.800 cM D11S902-D11S1310 interval (R. Ayyagari et al, unpublished observations). Several genes have been mapped to this smaller interval, including one that encodes a component of a voltage-gated potassium channel, KCNC1[26] (Fig 2). Potassium channel genes are important in neuronal function and are candidate genes for neural and neuromuscular diseases; however, no mutations in the KCNC1 coding sequence were detected in 5 USH1C patients. The sensitivity of SSCP analysis is 79% for single base substitutions in PCR products of approximately 200 bp and is much higher for deletions or insertions.[27] Results of heteroduplex analysis of known carriers also were normal.

Clinical tests to measure olfactory acuity did not identify significant differences between persons with USH1C and control populations. In both groups, an age-related decline in olfactory acuity occurs. Although this decline appeared to be greater in persons with USH1C, the difference was not significant ($p = .15$; Table 4). Plasma levels of PUFA and sarcosine also were not elevated in persons with USH1C.

In agreement with other studies, analysis of nasal cilia ultrastructure did demonstrate compound cilia. The pathologic significance of this finding is not known[28]; however, an association with infection has been proposed, as compound cilia are seen in a majority of maxillary sinus mucosal biopsies in persons with sinusitis,[29] while their incidence in mucosal biopsies of the inferior turbinate in infection-free controls is only 0.14% to 1.1%.[30-32] The number of compound cilia we observed in persons with USH1C was significantly higher than this range of reported normative values ($p < .0122$, *t*-test assuming unequal variances). Although all USH1C patients were endoscopically free of clinical evidence of sinusitis, because this test lacks specificity,[29] it will neither identify USH1C patients in the general population nor differentiate them from USH1A or USH1B patients.

## CONCLUSIONS

1. The USH1C gene is in linkage disequilibrium and is flanked by D11S902 and D11S1310. This finding may be useful to provide risk estimates to Acadians without a family history of USH1C.

2. There are no mutations in the KCNC1 coding sequence in persons with USH1C.

3. Persons with USH1C experience a normal age-related decline in olfactory acuity.

4. Both PUFA and sarcosine levels are normal in persons with USH1C.

5. The incidence of compound cilia in persons with USH1C is significantly higher than that in normal populations. This finding is unlikely to be helpful in the diagnosis of USH1C.

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## THIRTY-SECOND POSTGRADUATE COURSE IN EAR SURGERY

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*Ann Otol Rhinol Laryngol* 106:1997

# VESTIBULAR ABNORMALITIES IN CHARGE ASSOCIATION

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We report the vestibular abnormalities in 5 patients with the CHARGE association (Coloboma, Heart disease, Atresia of choanae, Retarded growth and development and/or central nervous system anomalies, Genital hypoplasia, and Ear anomalies). All patients had absent vestibular function as indicated by absent vestibulo-ocular reflexes and severe imbalance on simultaneous deprivation of proprioception and vision, as well as delayed motor development. All 6 semicircular canals were aplastic in each of the patients. While cochlear function was severely reduced in 6 of the 10 ears, it was absent only in 3 ears and was actually intact below 3 kHz in 1 ear. All 10 bony cochleas were present on computed tomography, and although 7 appeared abnormal, 3 appeared normal. This study confirms that absence of the bony semicircular canals in the presence of a bony cochlea is a characteristic finding in CHARGE association. It also demonstrates that these disproportionate structural abnormalities are reflected in the functional abnormalities: absent vestibular function with preservation of some cochlear function.

KEY WORDS — balance, CHARGE association, cochlea, hearing, inner ear anomaly, semicircular canal, vestibule, vestibulo-ocular reflex.

## INTRODUCTION

The association between coloboma and other congenital anomalies has been recognized since 1961.[1,2] Hall[3] reported 17 unrelated patients with choanal atresia and other multiple anomalies and suggested that these might constitute a new syndrome. Pagon et al[4] in 1981 reviewed 21 patients with coloboma, choanal atresia, and other, multiple anomalies and proposed the concept of the CHARGE association (Coloboma, Heart disease, Atresia of choanae, Retarded growth and development and/or central nervous system anomalies, Genital hypoplasia, and Ear anomalies). Diagnosis was arbitrarily based on having at least 4 of the 6 anomalies, including coloboma or choanal atresia. External ear anomalies and hearing impairment are common in patients with CHARGE association.[4-8] However, vestibular abnormalities, especially their physiologic and developmental aspects, have not been well described. Here we report vestibular abnormalities in 5 patients with CHARGE association from their clinicophysiological, developmental, and morphologic aspects and discuss their characteristics.

## PATIENTS AND METHODS

Five patients, 4 females and 1 male, from 3 to 23 years of age, with CHARGE association were seen with a parent at the Royal Alexandra Hospital for Children Neurology Clinic and at the Royal Prince Alfred Hospital Hearing and Balance Clinic. A relevant history was taken, and a neurologic and neurotologic examination was performed, including the following: 1) a stance and gait test with and without deprivation of vision (blindfold) and proprioception (soft foam rubber mat)[9]; 2) impulse tests of horizontal and vertical vestibulo-ocular reflexes[10]; and 3) postrotatory nystagmus test with Frenzel glasses after 10 revolutions.[11]

The oldest patient (patient 1) also attended the vestibular laboratory and had the following tests: 1) rotational tests using horizontal sinusoidal oscillations (peak velocity 50°/s) at 0.1 and 0.3 Hz, and constant acceleration at 40°/s per second for 5 seconds, and 2) caloric tests with water at 0°C, 30°C, and 44°C (40 seconds). All patients had temporal bone computed tomography (CT) scans.

## RESULTS

All patients met the criteria for CHARGE association as proposed by Pagon et al[4] (Tables 1[4] and 2). Every patient had a normal karyotype. There was no significant obstetric history for any patient.

*Patient 1.* This patient, a man 23 years of age, had a large coloboma of the left optic disc, and a smaller one on the right, below the disc. He had operations for cleft lip and palate; a right inguinal hernia was re-

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TABLE 1. SUMMARY OF SOMATIC ABNORMALITIES IN PATIENTS WITH CHARGE ASSOCIATION

| *Pt* | *Age* | *Sex* | *Coloboma* | *Heart Disease* | *Atresia of Choanae* | *Retarded Growth/CNS Anomalies* | *Genital Hypoplasia* | *Other Major Abnormalities* |
|---|---|---|---|---|---|---|---|---|
| 1 | 23 y | M | Yes | No | No | Yes/microcephalus | Yes | Cleft lip and palate, chest asymmetry |
| 2 | 9 y | F | No | ASD, VSD, PDA, PS | Left | Yes/Dandy-Walker syndrome | No | DiGeorge syndrome, hypoplastic lung, gastroesophageal reflux, FSHMD |
| 3 | 6 y 7 mo | F | Yes | PS | No | Yes/no | No | Cleft lip and palate, hypogammaglobulinemia, vesicouretic reflux |
| 4 | 5 y 7 mo | F | No | VSD, DORV | Left | Yes/no | No | Swallowing difficulty, left facial palsy |
| 5 | 3 y 4 mo | F | Yes | PDA | No | Yes/no | No | Swallowing difficulty, left facial palsy |

All patients met criteria for CHARGE association.[4] Anomalies of ears are not included in this Table (see Table 2).

ASD — atrial septal defect, VSD — ventricular septal defect, PDA — patent ductus arteriosus, PS — pyloric stenosis, FSHMD — facioscapulohumeral muscular dystrophy, DORV — double-outlet right ventricle.

paired at 2 months; and he also had operations for his low-set, cupped ears and for a left undescended testis. He was on testosterone treatment and was normally virilized. He had microcephalus and mild mental retardation and worked at a sheltered workshop. Other features included webbed neck, micrognathia, arachnodactyly, a hypoplastic left index fingernail with a mild flexion deformity of that digit, and a prominent right scapula due to chest asymmetry. On examination, he showed severe sensorineural hearing loss on the left and moderate mixed hearing loss on the right but normal cochlear function from 250 to 3,000 Hz (Fig 1A). A tympanogram in the right ear was type C, which suggested a retracted tympanic membrane.

He had crawled at 9 months and walked at 18 months. His gait was slightly wide-based and his tandem gait was poor, with increased sway and an exaggerated flexion of the toes. Although his stance on a mat with eyes open was good, he had difficulty with eyes closed. Cerebellar signs were absent. Vestibulo-ocular reflexes were absent on head impulse tests; postrotatory nystagmus was absent. On laboratory rotational tests he did not show any responses to sinusoidal oscillations or constant angular acceleration. Caloric responses were bilaterally absent.

Temporal bone CT (Fig 2) showed dilated vestibules with no semicircular canals, complete, normal cochleas with dilated basal turns, and normal middle ears. The vestibular aqueduct was enlarged on the right.

*Patient 2.* This patient, a 9-year-old girl, had features of both CHARGE association and DiGeorge

TABLE 2. SUMMARY OF VESTIBULOCOCHLEAR ABNORMALITIES IN PATIENTS WITH CHARGE ASSOCIATION

| | | | *Cochlear Function* | | *Vestibular Function* | *Computed Tomography* | | *Motor Development* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Pt* | *Age* | *Sex* | *Right* | *Left* | | *Cochleas* | *Canals* | *Sit* | *Crawl* | *Walk* |
| 1 | 23 y | M | Normal, 0.25 to 3 kHz | Severe loss, 80 dB to SO | Absent | R, L dilated basal turn | R, L absent | ? | 9 mo | 18 mo |
| 2 | 9 y | F | Total loss, SO | Total loss, SO | Absent | R dilated, L small | R, L absent | 3 y | 4 y 6 mo | 5 y |
| 3 | 6 y 7 mo | F | Severe loss, 50 to 80 dB | Severe loss, 75 to 90 dB | Absent | R, L small, complete | R, L absent | 12 mo | 3 y | Nil |
| 4 | 5 y 7 mo | F | Profound loss, 90 to 105 dB (ECochG) | Profound loss, 90 to 100 dB (ECochG) | Absent | R normal, L hypoplastic | R, L absent | 1 y 8 mo | 3 y | Nil |
| 5 | 3 y 4 mo | F | Total loss, SO (ECochG) | Profound loss, 80 to 100 dB (ECochG) | Absent | R Mondini, L normal | R, L absent | 11 mo | 1 y | 3 y |

Absence of semicircular canals is characteristic, and physiologic findings are consistent with morphologic findings. On other hand, cochlea tends to be preserved, and hearing of some patients was preserved to some extent.

SO — scale over, ECochG — evaluation with electrocochleogram, Nil — still incomplete at time of examination.

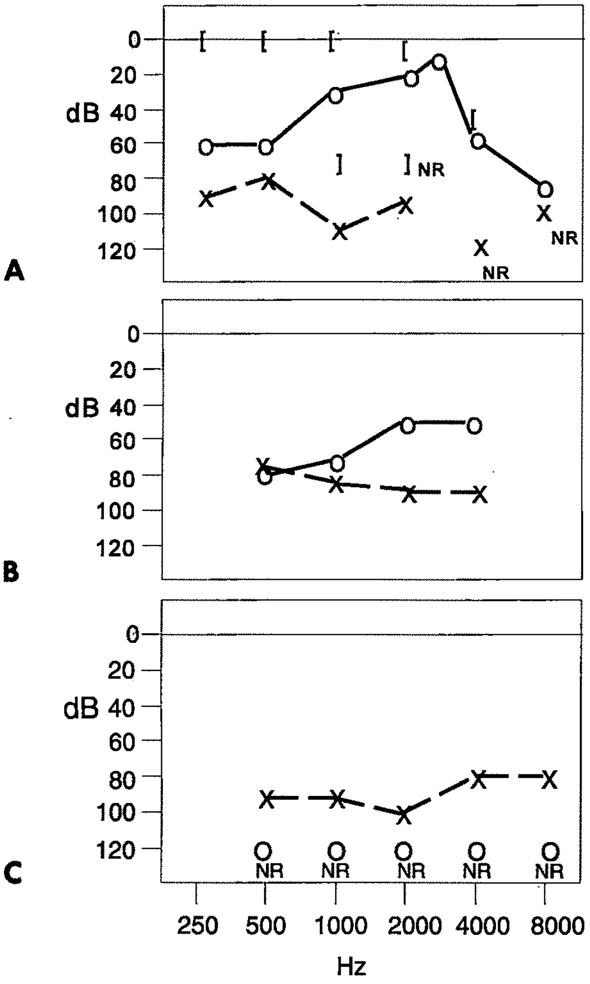

Fig 1. Audiograms. O — right air conduction threshold; X — left air conduction threshold; [ — right bone conduction threshold; ] — left bone conduction threshold; NR — scale over (no response at limit of audiometer). A) (Patient 1) Although hearing loss on left was profound, overall hearing loss on right was only moderate; below 3 kHz it was conductive and cochlear function was in fact normal. B) (Patient 3) Bilateral severe hearing loss, with right ear better than left. C) (Patient 5) Hearing level based on threshold of action potential to tone pips in electrocochleograms. Hearing was assessed to be totally lost on right and 80 to 100 dB hearing level on left.

syndrome[12]: congenital heart disease (atrial septal defect, ventricular septal defect, patent ductus arteriosus, and pulmonary stenosis), left choanal atresia (repaired), growth retardation (105 cm tall on growth hormone 14 units/wk), severe gastroesophageal reflux requiring a fundoplication, hypocalcemia, and mild immune deficiency, as well as hypoplastic lungs, asthma, left torticollis (released), a webbed neck, and mild facioscapulohumeral muscular dystrophy (which her mother also had), but no facial palsy. She had undergone a left hemicolectomy for necrotizing enterocolitis as a neonate. Her vision was normal. The pinnae were small, low-set, and cup-shaped. She showed bilateral total hearing loss.

She had sat at 3 years, crawled at 4.5 years, and walked independently at 5 years. Her gait was still wide-based but steady. She needed a flat surface and could not perform a tandem walk. There was complete lack of balance on a soft surface when blindfolded. Vestibulo-ocular reflexes were absent on head impulse testing; postrotatory nystagmus was absent. Cerebellar signs were absent. She communicated with about 100 different signs, and the degree of mental retardation was still unclear.

Temporal bone CT showed bilaterally absent semicircular canals, and a small left and a dilated right bony cochlea. Both middle ears were full of fluid. The mastoids were hypoplastic, with an osteolytic defect over the left lambdoid suture (most likely a dermoid cyst). The posterior fossa was hypoplastic, and this correlated with a hypoplastic cerebellar vermis and a Dandy-Walker variant on standard head CT.

*Patient 3.* This 6-year-old girl presented with bilateral colobomas of the retina, choroid, and optic nerves (visual acuity 6/30 on the right and 6/60 on the left), mild pulmonary stenosis, previously repaired cleft palate and cleft lip, growth retardation, previous low-grade vesicoureteral reflux, and gastrointestinal problems. Other problems included osteoporosis, immune deficiency (hypogammaglobulinemia), and chronic lung disease (asthma and recurrent aspiration in the past). Her ears were low-set and she had bilateral profound hearing loss (Fig 1B). She was fitted with bilateral ventilation tubes and hearing aids. She was of normal intelligence and spoke well.

Her motor development was delayed: she sat at 12 months and crawled at 3 years. On examination, she was not willing to walk more than 4 to 5 meters, and walked with a wide-based and stiff-legged gait. She needed a flat surface to stand, and her stance was even more unsteady on a mat. She had horizontal pendular nystagmus. Vestibulo-ocular reflexes were absent on head impulse testing; postrotatory nystagmus was absent. Cerebellar signs were equivocal.

Temporal bone CT showed bilateral small but complete bony cochleas, and absent semicircular canals. The ossicles were bilaterally hypoplastic.

*Patient 4.* This patient, a girl 5 years of age, had many features of CHARGE association: heart defects (double-outlet right ventricle, ventricular septal defect), left choanal atresia (repaired), growth retardation, poor swallowing and hiatus hernia (fed through gastrostomy up to 15 months, when fundoplication was performed), and bilateral low-set ears. She had bilateral profound hearing loss. She was fitted with ventilation tubes and hearing aids. She used a vibro-

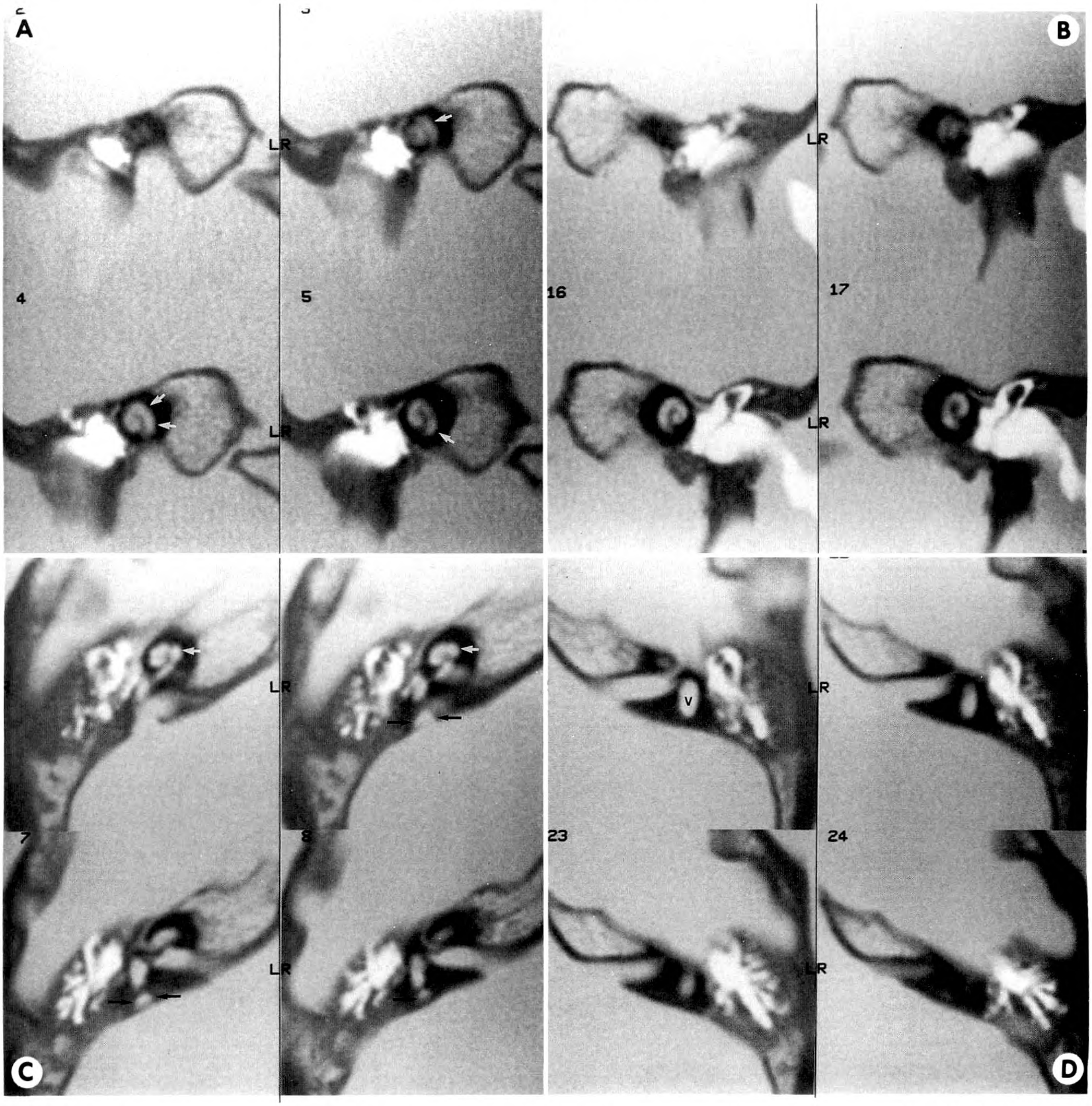

Fig 2. (Patient 1) A,B) Coronal and C,D) axial video-reversed temporal bone computed tomography scans. Semicircular canals are bilaterally absent, vestibule (V) is dilated on left (B,D), and vestibular aqueduct is dilated on right (A,C; black arrows). Bony cochlea is bilaterally present and appears normal, apart from basal turn on right (A,C), which appears to be slightly dilated (white arrows).

tactile aid and had some basic sign language. Her visual acuities were normal. As an infant she had a subarachnoid hemorrhage with apparently good recovery.

Play audiometry suggested that she had profound hearing loss. Thresholds by the compound action potential of the electrocochleograms were 100 dB hearing level (HL) to click on the left, 105 dB HL to click on the right, and 90 dB HL to 500-Hz tone pip in both ears.

It was not clear what degree of mental retardation existed, if any. She had sat at 20 months and crawled at 3 years. She shuffled on her bottom or used a walker. Her assisted gait was wide-based and stiff-legged. There was generalized hyporeflexia and hypotonia, and muscle biopsy showed a nonspecific increased variability in fiber size. There was a left facial paresis. Vestibulo-ocular reflexes were absent on head impulse testing; postrotatory nystagmus was absent. She could not walk at all on an uneven surface, and was so unsteady on a mat that a blindfold had little effect. There were no apparent cerebellar signs.

Temporal CT scans showed bilaterally absent semicircular canals. The right bony cochlea appeared normal, while the left appeared hypoplastic.

*Patient 5*. This patient, a 3-year-old girl, presented with bilateral choroidal coloboma affecting the optic discs and maculae. Her visual acuity bilaterally was 6/60. She had a patent ductus arteriosus (surgically closed), retarded development, a left facial palsy, and swallowing difficulties. Her pinnae were small, cup-shaped, and low-set.

She sat at 11 months, crawled at 1 year, and walked independently at 3 years. Her gait was still wide-based and stiff-legged. Vestibulo-ocular reflexes were absent on impulse testing; postrotatory nystagmus was absent. Stance testing was inconclusive because of poor cooperation.

Her hearing was assessed with electrocochleography. This revealed left severe and right total hearing loss. The threshold of the compound action potential to click was 80 dB HL on the right. She did not show any response to click on the left. On the basis of electrocochleograms by tone pip, her hearing was assumed to be 80 to 100 dB HL on the left and totally lost on the right (Fig 1C). She was fitted with ventilation tubes in both ears.

Temporal bone CT showed absent semicircular canals bilaterally. Other features included a dilated cochlea with absent apical turns (Mondini deformity)[13] and enlargement of the vestibular aqueduct on the right. The bony cochlea was normal on the left.

## DISCUSSION

Anomalies of the external, the middle, and the inner ear are common in CHARGE association.[4-8,14] Inner ear anomalies have been characterized by temporal bone histopathology[5,14] and by temporal bone CT.[6] Guyot et al[14] reported that the temporal bone histopathology of a patient with CHARGE association showed a Mondini deformity of the cochlea and the saccule, and complete absence of the semicircular canals and the utricle. Morgan et al[6] reported that of 26 CHARGE patients who had temporal bone CTs, most showed deformed or absent bony semicircular canals, and 12 also showed Mondini deformities of the cochlea. Wright et al[5] reported a patient with CHARGE association who showed absent bony semicircular canals and membranous semicircular ducts, although cochleas were present and inner and outer hair cells were identifiable. These reports all suggest that aplasia or severe hypoplasia of all the semicircular canals with presence of the bony cochlea might be specific to CHARGE association. All our patients showed the same type of inner ear anomaly.

Furthermore, this type of inner ear anomaly is rare except for CHARGE association; it was not found at all in a review of 63 patients with malformed ears.[15] However, it remains unclear whether this type of inner ear anomaly is strictly specific to CHARGE association. Parnes and Chernoff[16] reported 2 patients who appeared *not* to have CHARGE who nevertheless had bilateral semicircular canal aplasia with normal or near-normal bony cochleas on temporal bone CT. Furthermore, Hill et al[17] reported 2 patients with Kallmann's syndrome who had absence of semicircular canals but presence of the cochlea; these patients did not meet Pagon's criteria of CHARGE association, because they did not show choanal atresia or coloboma.

Suzuki et al[18] first reported that motor development was delayed in children with absent semicircular canals. Our report confirms that motor development is delayed in CHARGE children[5,8] (Table 1), presumably because of the absence of vestibular function. This report also confirms that children with congenital absence of vestibular function do gradually develop motor function, and presumably improve their equilibrium by using visual and proprioceptive inputs.[19]

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## FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

The Fourth Annual Golf Seminar — Advances in Otolaryngology–Head and Neck Surgery will be held March 11-16, 1997, at the Pinehurst Resort & Country Club in North Carolina. For information, contact Leslie Sullivan, Coordinator, University of Washington School of Medicine Virginia Merrill Bloedel Hearing Research Center, Box 357923, Seattle, WA 98195-7923; E-mail sullivan@u.washington.edu; telephone (206) 685-2962; fax (206) 616-1828.

*Ann Otol Rhinol Laryngol* 106:1997

# RHINOLITH: AN UNUSUAL CASE AND AN UPDATE

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An unusual case is reported in which a cleft palate patient with an oronasal fistula developed a true rhinolith. An update and discussion on rhinoliths are given.

KEY WORDS — cleft palate, rhinolith, update.

## INTRODUCTION

Nasal foreign bodies are not unusual, particularly among children, but true rhinoliths are very rare indeed.

Rhinoliths are completely or partially encrusted nasal foreign bodies, either exogenous or endogenous, depending on the origin of the nucleus on which encrustation occurs.[1]

Rhinoliths are considered endogenous if they arise around normal body material. These are usually closely related to the nasal region, such as misplaced teeth, sequestra, or dried blood clots that become lodged in the nasal cavity and form the nuclei of crystallization.

Rhinoliths are considered exogenous if non-human material or objects, usually unrelated to the nose and surrounding tissues (such as beads, buttons, fruit stones, cotton wool, or impression material, as in our case), gain access to the nasal cavity and act as the nidus for precipitation.

## CASE REPORT

A 49-year-old woman was referred by her general medical practitioner to the Department of Maxillofacial Surgery, St Lawrence Hospital, Welsh Centre for Burns, Plastic and Maxillofacial Surgery, Chepstow, Wales. She complained of blood-stained discharge from her left nostril over the previous 18 months, with increasing pain over the left side of her face, as well as an unpleasant taste from her palatal defect. Previous antibiotic therapy had improved her symptoms.

She was born with a cleft palate; she had three operations as a child but a palatal fistula still remained. An obturator was fitted after unsuccessful operations; this she wore for many years, until it was finally replaced by conventional complete upper and lower dentures 15 years prior to presentation. She had had no problem of regurgitation into the nose, and she had not seen her dentist since the dentures were fitted.

On extraoral examination, apart from pronounced fetor, no major abnormality was found. Intraoral examination revealed a fistula just to the left of the midline at the anterior third of the hard palate, roughly 6 to 8 mm in diameter (see Figure, A). There was communication through the defect with the nasal cavity, which was mostly occluded by a brownish gray, rough, hard, friable, and foul-smelling material. When the mass was probed through the hole, there was a copious foul-smelling, brownish, purulent, blood-stained discharge, and some gravel-like smaller fragments broke away. On anterior rhinoscopy some smaller fragments were removed orally and nasally. Then a brittle, calcified mass measuring about 2.5 × 1.5 × 1.5 cm was delivered through the nasopharynx. It was brownish black in color, with rough surfaces, and was extremely foul-smelling (see Figure, B).

Two weeks later all examinations, including indirect nasopharyngoscopy, showed no sign of foreign material or inflammation.

On detailed questioning, the patient was able to recall that when she had had dental impressions taken 15 years before, she had experienced some discomfort, a mild discharge, and breathing difficulty of which she complained at that time. Apart from reassurance, nothing further was done, and later, when the problems subsided, she forgot all about them. She did not relate this episode to her symptoms of nasal discharge.

Histopathologic examination of the removed specimens demonstrated a calcified body with an ill-defined laminated structure, suggesting that it may have enlarged by accretion of successional layers of

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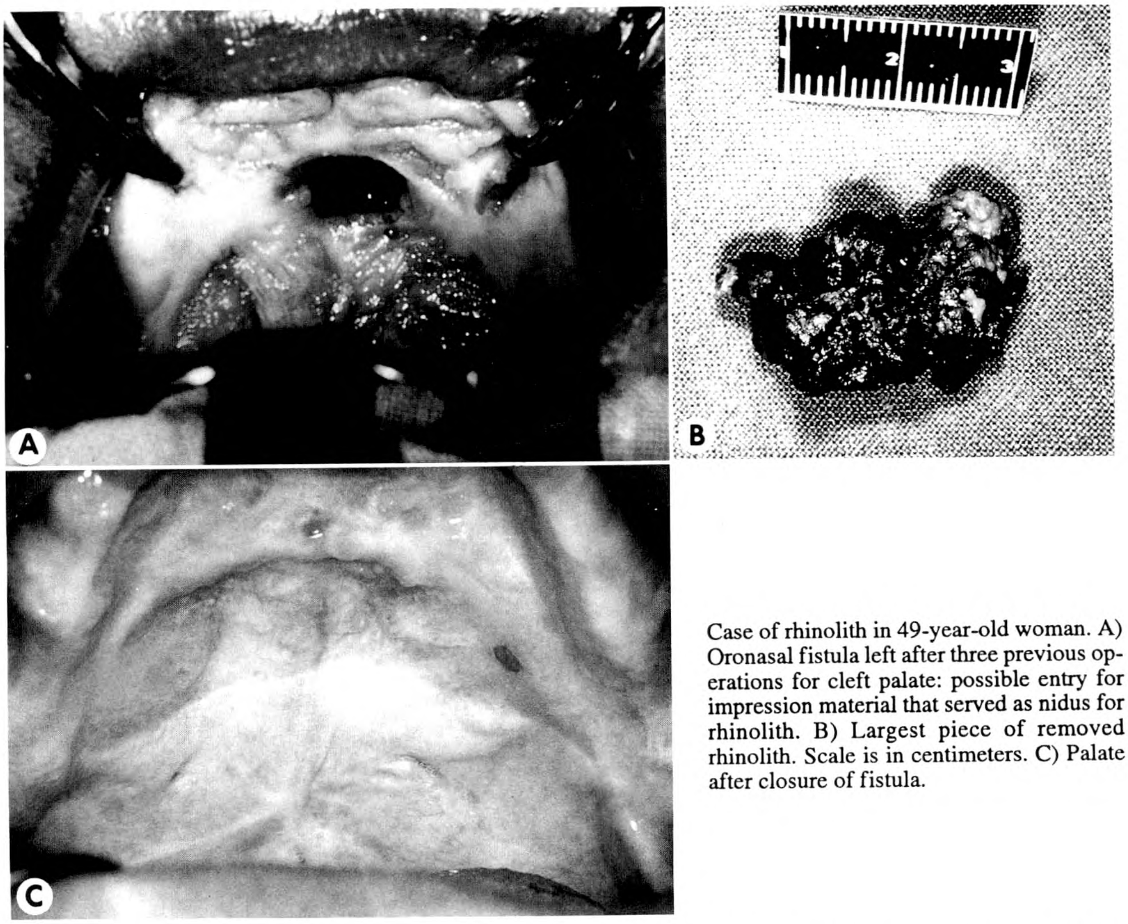

Case of rhinolith in 49-year-old woman. A) Oronasal fistula left after three previous operations for cleft palate: possible entry for impression material that served as nidus for rhinolith. B) Largest piece of removed rhinolith. Scale is in centimeters. C) Palate after closure of fistula.

material that had subsequently become calcified.

The residual palatal fistula was successfully closed in one procedure using local flaps in two layers after von Langenbeck[2] (see Figure, C).

## DISCUSSION

Foreign bodies are not unusual in the head and neck region, particularly in body orifices. Some are lodged at the time of accidents; others — and these are the majority — are inserted by hand, mostly by children. The variety of the retrieved objects (and the length of time they are tolerated by patients) is amazingly wide: impression materials,[3,4] tire valve,[5] eraser,[6] button,[7] paper,[8,9] oak tree twig,[10] button battery,[11] woodscrew.[12] Most frequent are beads, seeds, and fruit stones.[1,13]

On the other hand, true rhinoliths are exceedingly rare. They are the result of partial or complete encrustation of an intranasal foreign body by mineral salts precipitated onto them.

The most comprehensive study is still that by Polson,[1] who collected 384 cases over 440 years. With some refinement, his definition is still valid, although Bowerman[14] suggested the introduction of the maxillary antrolith as a separate entity. Further reports have been added to the previous cases.[5-7,14-24]

The cause and pathogenesis of rhinoliths are not fully understood, and the many speculations on the origin go back to Polson's monograph.[1]

It is thought that the predisposing factor is the entry and lodgment of a foreign body. However, other very important factors are needed, since only a tiny fraction of the foreign bodies become petrified.

It is necessary for the foreign material to evoke suppuration following acute and/or chronic inflammation. Further requirements are precipitation of salts (mostly calcium and magnesium), obstruction, and stagnation. The latter two prerequisites were underlined by Worgan's case,[17] which may have shed some light on the origin of endogenous rhinoliths. In a unilateral choanal atresia and subsequent infections, inflammations and fibrosis eventually produced a rhinolith, probably secondary to the choanal atresia.

Air currents, which probably help concentration and crystallization, are also needed. This was emphasized by Bowerman,[14] who pointed out that maxillary calcified bodies (antroliths) are extremely rare,[23,24] although foreign bodies in the antra are not uncommon. He also noted that no calculus had ever been reported in other paranasal sinuses. Although in recent years oro-antral fistulae have become less frequent than in the past, they are still not a rare occurrence, and it seems that this does not actually initiate the environment that is necessary for true calculus formation.

Time seems to be an important factor. It is not known how long is required for a foreign material to become encrusted, but most authors speculate in years. Allen[5] mentions an interesting case in which a tire valve was found in a boy's nose. On removal, crystallized deposits were already formed on its surface that probably would have become a rhinolith had it remained there longer unnoticed. No duration of stay was given, the boy having no recollection of when and how the valve became lodged. However, this case may shed some light on the phases of the formation of rhinoliths, with the first phase being crystalloid deposition.

The symptoms at the time of the lodgment are usually minor and are often long forgotten by the patient (as in our case). However, sooner or later most rhinoliths will make their presence known, although chance findings sometimes occur. The most frequent symptoms and signs after a latent period are unilateral nasal discharge (often blood-stained), obstruction and a feeling of blockage, epistaxis, slight to moderate swelling, headache, sinusitis, epiphora (rarely), and perforation of the nasal septum or palate.[5,15,19,21] It is probable that the stone and/or subsequent inflammation interferes with the sense of smell, because, as most authors emphasize, and as happened in our case, although the process produced a foul smell, the patient was totally unaware of it.

Defects of the palate, particularly developmental ones, are not uncommon, nor is dental impression-taking involving such patients. However, we found no reported case in which dental impression material acted as a possible nucleus for a true rhinolith as in our case.

The diagnosis of rhinolith may be suspected if there is a possible history of a foreign body, although this is rarely the case. Clinical findings and radiographs showing a calcified mass should raise the suspicion of a rhinolith. Computed tomography can be very useful.

The differential diagnosis includes any space-occupying lesion in the nose that gives the picture of a calcified mass on radiography, such as calcified nasal polyp, ossifying fibroma, odontoma, osteoma, osteosarçoma, chondroma, chondrosarcoma, or calcifying angiofibroma.

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ACKNOWLEDGMENTS — The authors thank Dr D. M. Walker and P. Ilori, Department of Oral Surgery, Medicine and Pathology, Dental School, University of Wales College of Medicine, Cardiff, for the histopathology; and D. Robinson, Consultant, ENT Surgeon at Queen Alexandra Hospital, Portsmouth, and Dr and Mr Hillams, Liverpool Dental Hospital, for their valuable advice.

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*Ann Otol Rhinol Laryngol* 106:1997

# SUMMATING POTENTIAL AND ACTION POTENTIAL GRADIENTS ON AND IN THE VICINITY OF THE ROUND WINDOW IN GUINEA PIG

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The purpose of this study was to determine the spatial gradients of summating potential (SP) amplitudes, action potential (AP) amplitudes, and SP/AP ratios for recording loci on the round window (RW) membrane and in its vicinity. Sixteen guinea pigs were tested by means of free-field click and tone burst (2.0 kHz) stimuli. Seven recording regions were specified: five equal-area regions on the RW membrane and two basal promontory regions. Statistically significant differences were found between the promontory regions and the RW membrane regions for the SP, AP, and SP/AP obtained with click stimuli, and for the SP and AP obtained with tone burst stimuli. The SP/AP ratio for tone burst stimuli did not differ significantly across the seven regions. The RW membrane was found to be isoelectric, but there were marked spatial gradients on the basal promontory. These results are of consequence for the interpretation of transtympanic electrocochleography recordings.

KEY WORDS — action potential, electrode position, guinea pig, round window, summating potential.

## INTRODUCTION

Electrocochleography (ECochG) is a technique of recording the stimulus-locked electrical responses of the organ of Corti and auditory nerve. It offers the clinician an electrophysiologic tool with which to evaluate patients with Meniere's disease, especially those with atypical histories, as well as cases of perilymphatic fistula.

The summating potential (SP) and action potential (AP) used in ECochG recordings are generated at different sites in the inner ear. Measurements of SP and AP can, therefore, be differentially affected by discontinuities of the conducting medium (as at some of the cochlear boundaries) and nonhomogeneities in conductivity (fluid versus bone). Thus, the SP and AP may not covary equally as the position of the recording electrode is altered. Van Deelen and Smoorenburg,[1] for example, showed that for a given stimulus, a significant change in SP and AP amplitudes occurred across the cochlear wall from apex to base.

The SP is a complex, multigenerator response with both positive and negative components that interact as a function of the intensity and frequency of the eliciting stimulus. The waveforms and amplitudes of the components are affected by the location of the recording electrode[2] and are quite sensitive to changes in cochlear status.[3] Goin et al[4] suggested that the SP response is highly sensitive to methodological variables. Several explanations of the large variability observed between individual animals were suggested, such as variations in electrode position, disparities in the anatomy of the middle ear, and differences in how a protocol is being executed by different members of a research team.[1] Such methodological variables must be of concern, since they may well affect the particular components of the complex SP visualized clinically.

In the present work, the SP and AP were systematically measured in each animal under carefully controlled conditions at numerous sites on the round window (RW) membrane and on the adjacent basal promontory. The purpose of this study was to determine the spatial gradients of the SP, the AP, and the SP/AP ratio for recording loci on the RW membrane and on the basal promontory.

## METHODS

Sixteen Hartley guinea pigs of both sexes weighing 350 to 450 g were used. Recordings were made from both right and left ears (N = 32). All animals had normal pinna reflexes and were free of middle ear infection as judged by direct observation.

For the ECochG recordings, the animals were anesthetized by intramuscular injection of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (8 mg/kg). Both middle ears were exposed from

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From the Department of Otorhinolaryngology, University of Texas Southwestern Medical Center (Badr-El-Dine, Meyerhoff), and Callier Center for Communication Disorders, University of Texas at Dallas (Gerken), Dallas, Texas. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Texas Southwestern Medical Center at Dallas.

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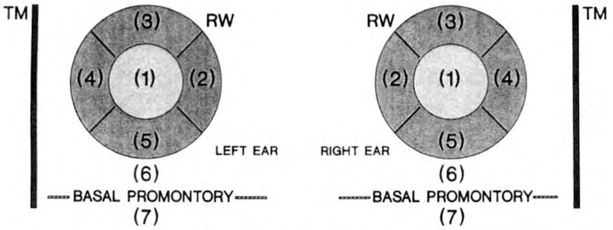

Fig 1. Diagram showing seven regions monitored in both right and left ears. Regions 1 through 5 are on round window membrane, and regions 6 and 7 are on basal promontory. TM — tympanic membrane.

a dorsolateral approach. The exposure allowed complete visualization of the RW and the adjacent bony cochlear wall without injuring the tympanic membrane or the ossicular chain. A warm thermal blanket under the animal maintained body temperature within 36.5°C to 37.5°C. Supplementary doses of anesthetic were used throughout the procedure.

The recording electrode was a Teflon-coated 75-µm stainless steel wire that was electrolytically tapered and rounded. The electrode was positioned with a micromanipulator by means of an operating microscope (Zeiss, OpMi-1). In each animal, recordings were made from numerous sites on the RW membrane and on the ventral aspect of the promontory within 3 mm from the rim of the RW membrane. A stainless steel reference electrode was placed on the denuded neck muscles. The recording sequence included repeated measures at specific sites to monitor response consistency. Care was taken to keep the RW membrane intact and to keep fluid accumulation to a minimum by wicking as required. The experiments were conducted in an electrically and acoustically shielded room.

Stimuli were presented free-field by a driver (Altec, model 288C) connected to an exponential horn (Altec Lansing, model MR II 594). Because the ears of the guinea pig were centered in the plane of the mouth of the horn, there was approximately a 1-millisecond (ms) acoustic delay relative to the electrical signal. Condensation and rarefaction clicks were 0.1 ms in duration and 105 dB peak sound pressure level (pSPL). Tone bursts were 2.0 kHz, 105 dB SPL, with a 5-ms plateau, 1-ms rise and fall times, and a 0° or 180° phase.

The ECochG potentials were recorded by means of an amplifier (Grass P511K) with a gain of 5,000 and with high-pass and low-pass filter settings of 10 Hz and 3 kHz, respectively. The potentials were averaged over 100 sweeps. Averaging was begun 2 ms before the presentation of a stimulus in order to provide a baseline for the measurement of the ECochG potentials. For the click stimulus, the amplitude of the SP was measured from the prestimulus baseline to the peak of the deflection preceding the AP. When the SP showed two components, ie, a biphasic SP, both the positive SP (+SP) and the negative SP (−SP) were taken into consideration so that SP was measured from the +SP peak to the −SP trough. For the tone burst stimulus, the SP amplitude was measured from the prestimulus baseline to the plateau portion of the stimulus between 5 and 6 ms (re the electrical signal). For both clicks and tone bursts, the AP amplitude was measured from the prestimulus baseline to the negative peak, N1.

For data analysis, the RW membrane was divided into five regions of equal surface area as shown in Fig 1: a circular central region surrounded by four quarter-circle regions. In addition, the promontory ventral to the rim of the RW was divided into two regions: one within 1.5 mm of the RW and the second covering between 1.6 and 3.0 mm of the RW rim.

For statistical evaluation of the data, the SAS General Linear Model (GLM) was used.

## RESULTS

The results of this study were based on a total of 409 observations over the seven recording regions. The SP and AP potentials were clear and replicable at all electrode positions, although as detailed below, the amplitudes were significantly larger for the RW membrane locations as compared to the promontory recording sites.

The Table shows the mean amplitudes, based on 32 ears, of the SP and AP potentials, as well as the SP/AP ratios for both click and tone burst stimuli on the RW membrane (regions 1 through 5) versus the promontory locations (regions 6 and 7). The mean SP

COMPARISON OF ROUND WINDOW AND PROMONTORY RECORDINGS

| | *Click Stimulus* | | *Tone Burst Stimulus* | |
|---|---|---|---|---|
| | *RW Membrane (Regions 1-5)* | *Promontory (Regions 6 and 7)* | *RW Membrane (Regions 1-5)* | *Promontory (Regions 6 and 7)* |
| SP | 58.31 ± 53.1 | 21.66 ± 23.9 | 59.62 ± 37.1 | 42.59 ± 22.5 |
| AP | 576.6 ± 123.5 | 380.7 ± 99.6 | 308.9 ± 96.6 | 184.6 ± 52.2 |
| SP/AP | 0.10 ± 0.10 | 0.06 ± 0.06 | 0.23 ± 0.14 | 0.23 ± 0.11 |

Data are mean ± SD.
RW — round window, SP — summating potential, AP — action potential.

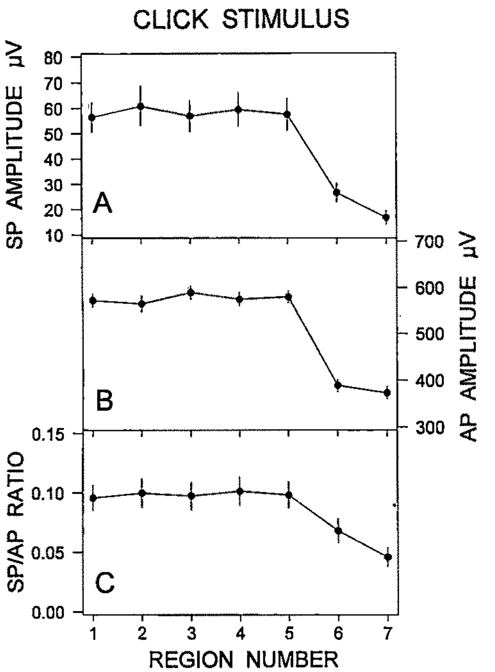

Fig 2. Amplitudes of click-evoked electrocochleography potentials. Error bars represent ±1 SEM. Results are based on 32 ears. A) Plot of mean summating potential (SP) amplitudes versus region number for electrode location (see Fig 1). B) Plot of mean action potential (AP) amplitudes. C) Plot of SP/AP ratios.

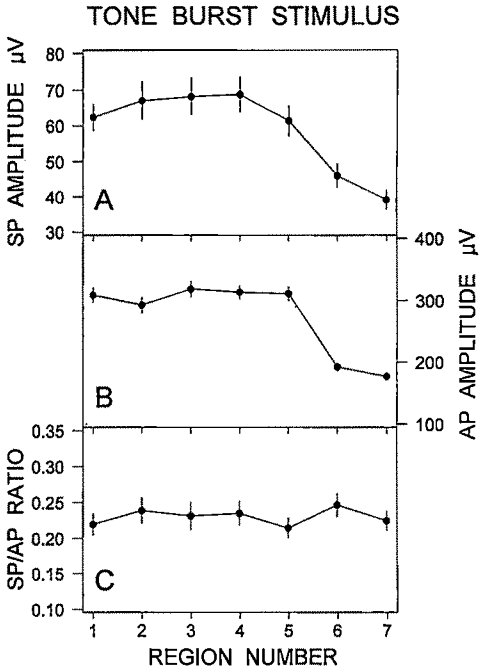

Fig 3. Amplitudes of tone burst (2.0-kHz)–evoked electrocochleography potentials. Error bars represent ±1 SEM. Results are based on 32 ears. A) Plot of mean SP amplitudes as in Fig 2. B) Plot of mean AP amplitudes. C) Plot of SP/AP ratios.

amplitudes recorded over the RW membrane for click and tone burst stimuli were 58.31 and 59.62 µV, respectively; while over the basal promontory the corresponding amplitudes were 21.66 and 42.59 µV. Similarly, the mean AP amplitudes recorded over the RW membrane for click and tone burst stimuli were 576.6 and 308.9 µV, respectively; while over the basal promontory the corresponding amplitudes were 380.7 and 184.6 µV.

The relatively high standard deviations (see Table) are due to the high interanimal variability of all measures despite the use of carefully standardized test procedures. Intra-animal variability, however, was very low.

The SP/AP ratios for clicks decreased markedly, from 0.10 on the RW membrane to 0.06 over the basal promontory locations. For the tone burst stimulus, there was little or no difference between the SP/AP ratios on the RW membrane or on the basal promontory, irrespective of the electrode position: the mean SP/AP ratio for all seven regions was 0.23.

The mean amplitudes of the click-evoked ECochG potentials in relation to the seven regions monitored are shown in Fig 2. Figure 2A shows that the mean click SP amplitudes were relatively stable across the five RW membrane regions (regions 1 through 5), while a large decrease in amplitude occurred over the two promontory regions (regions 6 and 7). Figure 2B shows the relative stability of the mean AP amplitudes over the five RW membrane regions and a marked decrease in amplitude over the two promontory regions. The SP/AP ratios for click stimulus versus regions, as shown in Fig 2C, were stable in all regions of the RW membrane, but decreased as the electrode was moved away from the RW membrane. This decrease in SP/AP ratios indicated that there was not a proportional decrease in SP and AP amplitudes. Both SP and AP potentials and the SP/AP ratios differed significantly across the seven regions (SAS GLM, p = .0001). Use of the Tukey option with the GLM showed that the differences were entirely between the two promontory regions and the five RW membrane regions. There were no significant differences within the five RW membrane regions or within the two promontory regions.

The mean amplitudes of tone burst–evoked ECochG potentials in relation to the seven regions monitored are shown in Fig 3. Figure 3A shows the mean SP amplitudes across the five RW membrane regions and the two promontory regions. There was little variation in the SP measures over the RW membrane, but there was a marked decrease of SP amplitude over the two promontory regions. Figure 3B shows the relative stability of the mean AP amplitudes across the five RW membrane regions and a marked decrease in amplitude over the two promontory regions. Both SP and AP potentials differed significantly across the seven regions monitored (SAS

GLM, p = .0001). Use of the Tukey option with the GLM showed that the differences were entirely between the two promontory regions and the five RW membrane regions. There were no significant differences within the five RW membrane regions or within the two promontory regions. In contrast to the results obtained with the click stimulus, the SP/AP ratio for the tone burst stimulus (Fig 3C) was quite stable and did not differ significantly across the seven regions (p = .06). This stability indicated that proportional changes occurred in the SP and AP amplitudes across the seven recording sites.

## DISCUSSION

The guinea pig cochlea consists of a spiral canal, 18 mm in length, encompassed in 3.25 turns. The cochlea, throughout its length, projects into the bulla just medial to the tympanic membrane and the handle of the malleoincudal complex. The RW membrane lies widely exposed in a horizontal plane that is perpendicular to the plane of the oval window.[5] On the other hand, the human cochlea consists of a 32-mm-long spiral canal encompassed in 2.5 turns. The cochlea is much more completely encased in the petrous bone. The promontory is formed mainly by the basal turn of the cochlea facing the middle ear cavity, and the RW niche is angled posteroinferiorly with respect to the external auditory canal. The RW membrane frequently lies partly hidden behind an incomplete curtain of mucosal fold. The bony lip of the RW niche usually has to be removed if the RW membrane is to be widely visualized.[6]

The differences between the anatomy of the cochlea in humans and in guinea pigs, as they relate to electrophysiology, were discussed by Eggermont[7] and Van Deelen and Smoorenburg.[1] For transtympanic ECochG recording in humans, in contrast with that in guinea pigs, only a very restricted portion of the bony surface of the cochlea can be used. Thus, the intratympanic extracochlear ECochG potentials obtained from homologous recording loci in humans and guinea pigs are not necessarily directly comparable, because of the anatomic differences.

Electrophysiologic differences between guinea pig and human cochleas may be related to anatomic factors, as well as to species-related functional factors. For example, Van Deelen et al[8] observed in guinea pigs an increase in the SP/AP ratio due to hydrops with an apical electrode, but not with an RW electrode. They suggested that in guinea pigs an RW recording may not be optimal for the demonstration of a hydropic effect. Yet, the use of apical electrode positions does not replicate the recording conditions in humans. Practically and anatomically, apical electrode positions are not feasible for human recording.

The questions raised in the present study are relevant to clinical ECochG recordings, but the answers cannot be obtained in human subjects. Consequently, for the present study, keeping in mind the specific anatomic differences between guinea pigs and humans, the recording locations in the guinea pig were chosen to simulate as much as possible the recording conditions in humans, whether in clinical testing or during intraoperative monitoring. Use of regions 1 through 5 on the RW membrane in guinea pigs simulated the recording electrode placed directly against the RW membrane during intraoperative monitoring in humans,[9] while regions 6 and 7 over the basal promontory in guinea pigs paralleled the transtympanic promontory recording in human clinical testing.[10]

The SP and AP amplitudes showed similar patterns of change as a function of electrode location for either the click or 2.0-kHz tone burst stimuli. There was, however, a marked contrast in spatial gradients of the SP/AP ratios for the two stimuli. In order to account for the SP, AP, and SP/AP results, the distribution of the sources of SP and AP and the relative distances of the sources from the recording electrode must be taken into consideration. The factors of source distribution and source distance are discussed below.

Stimulus spectrum determines the region of the cochlea that is maximally activated. In regard to the spectrum of the click stimulus, the click was generated by a driver with significant output above 20 kHz. Thus, the click stimulus must be considered as a true high-frequency stimulus. For the click stimulus, an electrode positioned on the RW membrane or on the basal promontory would have been in close proximity to the generators of the SP and AP potentials. Thus, large and replicable responses were obtained for the click stimulus in all seven regions, but there were steep response gradients between the RW membrane and promontory loci. This is in agreement with Ruth et al[10] and Staller.[11] For the click stimulus, the decrease in SP/AP ratio in the promontory locations meant that there was a greater attenuation of the SP amplitude relative to the AP amplitude as the electrode was moved from the RW membrane to the promontory. The basis for the unequal attenuation of the SP and AP, therefore, would seem to be related to the close proximity of the sources to the recording electrode, a condition that emphasizes local field asymmetries and nonhomogeneities in the conducting media.

In the same vein, it is likely that the low-frequency (2.0-kHz) tone burst stimulus activated generators that were distributed over more apical regions of the cochlea. These low-frequency generators, as seen from the RW membrane or from the basal promontory locations, were more remote from the recording electrode than the generators associated with the response to the click stimulus. Thus, small changes in electrode position would have relatively less effect than when high-frequency stimuli were used. This resulted in more equal percentage decrements in both SP and AP amplitudes as the electrode was moved from the RW membrane to the promontory. Consequently, for the tone burst stimulus, the more equal attenuation of SP and AP yielded relatively stable SP/AP ratios across all recording locations. Thus, the effect of change in electrode position on the SP/AP ratio differed between click and tone burst stimuli principally because of physical considerations relating to the spatial distribution of the SP and AP electric fields. In summary, the nearer the electrode to the generator site, the clearer and more replicable the response, but the greater the effect of electrode position. In a comparison between transtympanic and extratympanic ECochG recording in humans, as suggested by Elberling[12] and confirmed by Mori et al,[13] the contributions from different cochlear generators showed more variation with electrode position in transtympanic recordings (ie, more proximal to the generators) than in extratympanic recordings (ie, more distal to the generators). Therefore, by extrapolation, our findings corroborate those of Elberling[12] and Mori et al.[13] In this regard, ear canal recording may prove more valid even though signal strength is reduced.

Another possible explanation for the different behavior of the SP/AP ratios is suggested by the noise bias concept.[14] The noise bias explanation, as proposed by Coats,[14] is based on the idea that the SP and AP signals are equally attenuated, but are superimposed on a constant-amplitude noise. The extent to which potential amplitudes are subject to bias depends on the amplitudes of the potentials relative to the background noise. The noise would exert a greater bias effect on smaller signals. Mori et al[13] found that although the signal-noise ratio was smaller (ie, poorer) with the extratympanic method than with the transtympanic method, the former procedure increased the intertest reliability. In our experiment, however, the noise component of any of the signals was minimal; hence, there was little, if any, opportunity for alteration of signal amplitudes.

A further point of importance is that the RW membrane was, to a first approximation, *isoelectric* with respect to SP and AP amplitudes. Consequently, SP/AP ratios were stable as well. This is probably due to the thinness and uniformity of the RW membrane, which is in direct contact with a homogeneous and isotropic medium, the perilymph. With more measurements and with a greater number of regions, however, edge effects near the rim of the membrane may become apparent.

In animal recordings, the SP may be positive or negative, depending on the electrode recording site in the bulla and on the physiological condition of the ear.[3,4] In our recordings, the SP polarity for the 2.0-kHz tone burst stimulus was always positive. For the click stimulus, the SP generally was positive, but sometimes showed a biphasic waveform. Even so, the fact that SP polarity was not necessarily consistent across all electrode positions indicated that the variations in polarity observed were a function of location of the electrode rather than a change in the generator itself. This is in accord with Kanzaki et al[2] and Campbell et al.[15]

## CONCLUSIONS

On the basis of the above results, we conclude that the RW membrane can be considered isoelectric for the SP and AP. Steep electrical gradients may be encountered, however, if the electrode is moved away from the RW membrane. The exact nature of the gradient depends on the spectral content of the acoustic stimulus.

Clinically, the significant variations in the SP and AP measures, particularly in the SP/AP ratio for clicks, are of consequence for the interpretation of human transtympanic ECochG recordings. Large ECochG potentials, while desirable with respect to signal to noise ratio, may be subject to significant variability due to spatial, rather than noise, factors.

ACKNOWLEDGMENTS — The authors thank C. G. Wright, PhD, and K. S. Robinson for their continuous support and help.

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*Ann Otol Rhinol Laryngol* 106:1997

# GLUCOSE UTILIZATION OF THE RAT VESTIBULAR END ORGANS: A QUANTITATIVE 2-DEOXYGLUCOSE STUDY

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The local metabolic rate of glucose utilization (LMRglc) for the rat vestibular end organs was determined with a modification of the [$^{14}$C]deoxyglucose method. Data are expressed as micromoles per 100 g per minute ± SEM. Results indicate that the LMRglc is similar within the utricle (40.3 ± 3.2) and saccule (41.2 ± 5.5) and significantly higher than that for the superior (20.1 ± 2.9), posterior (25.4 ± 2.0), or lateral canal (22.0 ± 2.6) ampullae. These differences in LMRglc may be related to differences in the ratios of sensory to nonsensory cells, dark cell distributions, response to acoustic stimulation, or activity levels during the experimental period. Given the high blood flow rates reported for the vestibular end organs by Lyon and coworkers, a much higher LMRglc was expected. Together, these data would suggest that delivery of metabolites is not a primary regulating force for vestibular blood flow. Instead, the primary reason for a high blood flow rate may be waste removal, the maintenance of pH, ion balance, and/or temperature.

KEY WORDS — deoxyglucose, inner ear, labyrinth, metabolism, rat.

## INTRODUCTION

The deoxyglucose (DG) method for determination of local rates of glucose metabolism (LMRglc) has been used extensively to study regional brain responses to physiological, pharmacological, and behavioral perturbations.[1] This technique has also been used by several laboratories to provide some qualitative data on cochlear metabolism[2-6] and has contributed to our present understanding of metabolic activity of the cochlea. While there are a few studies describing the metabolism of the vestibular end organs,[7] there have been no quantitative investigations, and our knowledge of the metabolic processes within the vestibular system is essentially nonexistent. Since the pathophysiology of several diseases afflicting vestibular function has been postulated to reflect alterations in the cellular metabolism, a baseline understanding of the metabolic activity in the healthy state would be valuable.

The goal of the present study was to establish, quantitatively, the LMRglc of the individual vestibular end organs — specifically, the saccule, utricle, and individual ampullae of the semicircular canals in the healthy rat. Results show that the LMRglc values of the utricle and saccule are similar and significantly higher than those of the superior, posterior, or lateral semicircular canal ampullae.

## METHODS AND MATERIALS

A total of 11 healthy male Fischer 344 rats (260 to 315 g; 5 months old) provided useful data. Each animal was anesthetized with halothane and both femoral arteries and veins were cannulated with PE 50 tubing (Clay Adams, Sparks, Md). One arterial catheter was used for constant blood pressure monitoring (Coulbourn T42-20 pressure transducer connected to an S72-25 transducer coupler type A strain gauge bridge, an L25-12 LabLinc 12 bit analog input module interfaced to an IBM PC with a DATAQ A-D board, and software). The other arterial line was used for obtaining blood gas samples and blood samples during the experimental period. The caudal portion of the animal was immobilized by applying a loose plaster cast from mid-thorax.

The LMRglc was determined by a modification of the technique described by Sokoloff et al.[8] Through one venous catheter a bolus of [$^{14}$C]-2-deoxyglucose (250 μCi/kg; DuPont-NEN, Wilmington, Del, #NEC720) was injected into the awake animal (at least 2.5 hours after anesthesia) at time zero. Timed arterial blood samples (50 to 75 μL) were taken throughout the 45-minute experimental period as follows: at 0 time (the time of the tracer injection) blood was sampled continuously for 30 seconds followed by samples at 0.75, 1, 1.5, 3, 5, 7.5, 10, 15,

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From the Departments of Otolaryngology and Communication Sciences (both authors) and Anatomy and Cell Biology (Lyon), State University of New York, Health Science Center, Syracuse, New York. Supported by a Hendricks Foundation Grant (M.J.L.). This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the State University of New York, Health Science Center at Syracuse.

Some of the data within this manuscript have been published in abstract form: Association for Research in Otolaryngology Midwinter Meeting, St Petersburg Beach, Florida, February 6-10, 1994, and New York Academy of Sciences, New Directions in Vestibular Research, New York, New York, June 25-27, 1995.

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20, 25, 30, 35, 40, and 45 minutes (blood sample total 1 to 1.5 mL). After the last blood sample, the animal was painlessly sacrificed by lethal Nembutal injection and decapitated, and the temporal bones were rapidly removed and immediately frozen in isopentane cooled with dry ice. As a control for the technical modifications, in 3 animals the inferior colliculi were also dissected free and bisected, and each side was frozen separately in cooled isopentane.

The blood samples were centrifuged and a 20-µL plasma sample was taken for scintillation counting in a Packard A4600 liquid scintillation counter (Packard Instrument Company, Downers Grove, Ill), and glucose determinations were made with a Beckman II Glucose Analyzer (Beckman Instruments Inc, Fullerton, Calif).

The temporal bones were freeze-dried for a minimum of 36 hours and the individual end organs were then dissected free and digested in 300 µL of 3N sodium hydroxide. Similar end organs from the right and left temporal bones were pooled together for individual animals. After digestion was complete, 300 µL of 3N hydrochloric acid was added and two 100-µL aliquots were removed for protein assay. The assay was performed by the method of Lowry et al[9] (Sigma Diagnostics, St Louis, Mo). The remaining portion of the specimen was mixed with Hionic-Fluor liquid scintillation cocktail and the activity was determined. Since the high concentration of sodium hydroxide interfered with the protein assay, end organs from 9 additional animals were removed and homogenized and protein assays were performed. The mean protein concentration for each end organ was then used in the calculations.

Cryostat sections, 20 µm, of one randomly chosen inferior colliculus from each animal were collected on coverslips and dried on a hot plate at 60°C for a minimum of 5 minutes. Every fifth section was placed serially into X-ray cassettes containing OM1 X-ray film (Eastman Kodak Co, Rochester, NY) and a set of [$^{14}$C]-methylmethacrylate standards that included a blank and a series of increasing known [$^{14}$C] concentrations. Adjacent sections were collected and stained for histologic verification of the structures being examined in the autoradiographs. After 3 to 5 days of exposure, the film was developed. Concentrations of [$^{14}$C] in the inferior colliculus were determined by image analysis of the autoradiographs (M2, Imaging Research Inc, St Catharines, Canada). Values for the LMRglc on the whole colliculus (not subdivided into nuclei) were calculated by the operational equation of the DG method.[8] The LMRglc is the mean value of 15 to 20 sections. These data were compared to the data obtained for the opposite side, which was processed as described for the end organs, and the LMRglc was calculated as delineated below.

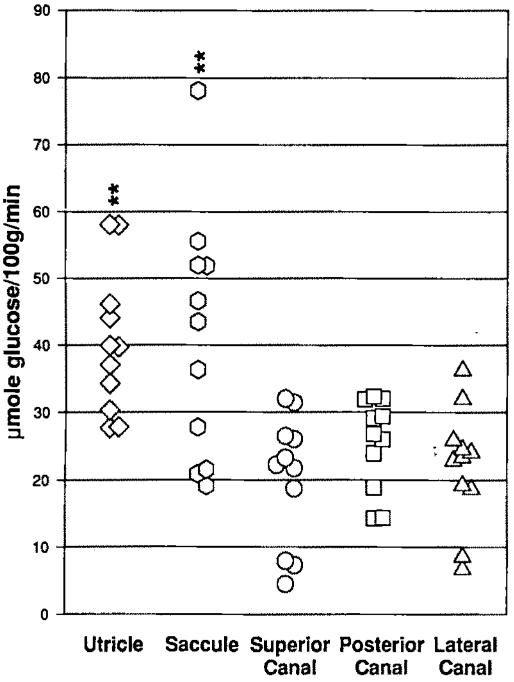
In order to calculate the LMRglc for the individual vestibular end organs, it was necessary to make several assumptions. First, the lumped constant in Sokoloff's operational equation is considered to be the same for the vestibular end organs. It is not possible to determine the lumped constant for the vestibular system, since one of its requirements is the measurement of arterial and venous concentrations of DG. As stated in previous work,[4] even if differences between the arterial and venous sides of the inner ear were measurable, the lumped constant would reflect the combined metabolism of the whole cochlea and vestibular system. Second, the protein concentration of a gram of wet weight brain is assumed to be approximately 100 mg.[10] Third, a gram wet weight of vestibular end organ is considered to contain the same amount of protein as a gram wet weight of brain. On these assumptions, it is then possible to calculate the LMRglc from the equation

$$\mathrm{LMRglc} = \frac{\mathrm{Ci}^* - \mathrm{G5}}{\mathrm{LC} \times \mathrm{U4} \times t \times 0.18}$$

where Ci* represents the measured radioactivity per microgram of protein in end organ, G5 is the final tissue DG activity, LC is the lumped constant derived by Sokoloff et al,[8] U4 is the integrated tissue specific activity, t is time, and 0.18 represents the molecular weight of glucose per micromole. The results are in micromoles per milligram of protein per minute, which is then converted to micromoles per 100 g per minute, the conventional units of expression for LMRglc.

Since this was the first time that the DG technique had been used quantitatively in studies of the peripheral vestibular system, it was necessary to verify that the level of glucose-6-phosphatase (G-6-P) activity in the vestibular system is at a level similar to that found in the brain. The measurement of LMRglc is dependent on low levels of G-6-P, since this enzyme can dephosphorylize the DG and allow it to reenter the precursor pool, leading to an underestimation of the LMRglc. Verification was done by the techniques of Baginski et al[11] to measure liberated phosphate. Samples of brain (extremely low levels of G-6-P), liver (high levels of G-6-P), and the vestibular end organs from the same animals were used. The tissue assays were all done in duplicate along with a standard, a blank containing no tissue sample, and a control in which the tissue sample was added following the blocking step.

Data were statistically analyzed with a one-way analysis of variance (BMDP, P7D Statistical Soft-



Glucose utilization for individual vestibular end organs. Double asterisks denote statistically significant difference from superior, posterior, and lateral semicircular canal ampullae.

ware, Los Angeles, Calif). Post hoc analysis was done with Tukey's Studentized range method. A probability of $p < .05$ was selected as the level for accepting or rejecting the null hypothesis.

## RESULTS

All data are expressed as mean ± SEM except where indicated. Arterial blood pressure monitoring demonstrated consistent mean arterial pressures between animals of 114.8 ± 1.9 mm Hg. The blood chemistry data were also well within normal limits, indicating that the animals had tolerated the surgical procedures and casting well and were not under any stress (hemoglobin 18.5 ± 0.68%; pH 7.374 ± 0.009; $P_{CO_2}$ 35.68 ± 2.66 mm Hg; $P_{O_2}$ 108 ± 6 mm Hg). Analysis of [$^{14}$C]-DG plasma curves showed the typical early peak with good clearance over the subsequent 45 minutes.

The amount of phosphate liberated by the activity of G-6-P from samples containing similar protein concentrations was 0.0000 µmol for brain and each of the vestibular end organs and 9.32 ± 0.89 µmol for the liver. Results from inferior colliculus autoradiographs showed the typical density patterns for the nuclear subgroups as previously reported.[12] By standard densitometry techniques, the LMRglc for the whole inferior colliculus was 71.25 ± 1.38 (SD) µmol/100 g per minute. Data obtained with modified techniques (72.93 ± 6.24 µmol/100 g per minute) were not different. These control data demonstrate that the modifications and assumptions that were made are valid.

Data (micromoles per 100 g per minute) from the individual vestibular end organs indicate differences in the LMRglc. The LMRglc values for the utricle (40.3 ± 3.2) and saccule (41.2 ± 5.5) were similar and significantly higher than those for the superior canal (20.1 ± 2.9), posterior canal (25.4 ± 2.0), or lateral canal ampulla (22.0 ± 2.6; see Figure). Raw data for the individual animals are presented in the Table.

## DISCUSSION

The LMRglc in the inner ear is a reflection of cellular metabolism and may ultimately be found to play a role in various disease processes. Qualitative studies of the LMRglc of the cochlea have been performed.[2-6] Usually, these studies compared the differences between right and left sides by means of autoradiographic densities. These qualitative data are useful to some extent and have provided informa-

LOCAL RATE OF GLUCOSE METABOLISM FOR INDIVIDUAL VESTIBULAR END ORGANS

| *Animal* | *Utricle* | *Saccule* | *Superior Canal* | *Posterior Canal* | *Lateral Canal* |
|---|---|---|---|---|---|
| 1 | 39.75 | 78.19 | 7.89 | 31.94 | 19.23 |
| 2 | 39.96 | 51.89 | 22.21 | 32.02 | 22.87 |
| 3 | 27.64 | 21.52 | 4.46 | 14.35 | 6.64 |
| 4 | 37.06 | 52.00 | 26.46 | 29.09 | 31.99 |
| 5 | 46.12 | 27.71 | 21.67 | 26.70 | 25.84 |
| 6 | 58.00 | 55.58 | 25.97 | 25.95 | 23.33 |
| 7 | 34.30 | 36.30 | 23.18 | 23.87 | 18.67 |
| 8 | 30.31 | 46.63 | 32.00 | 29.38 | 24.03 |
| 9 | 58.09 | 20.89 | 18.70 | 18.89 | 24.48 |
| 10 | 27.77 | 19.14 | 7.26 | 14.31 | 8.55 |
| 11 | 44.08 | 43.58 | 31.43 | 32.34 | 36.24 |
| Mean ± SEM | 40.28* ± 3.22 | 41.22* ± 5.50 | 20.11 ± 2.90 | 25.35 ± 2.04 | 21.99 ± 2.64 |

Data are in micromoles per 100 g per minute.

*Statistically significant difference from superior, posterior, and lateral semicircular canal ampullae.

tion on the metabolism of these tissues. While these studies have been done on the cochlea, there are no data for the LMRglc of the peripheral vestibular system. The study presented here provides the first such data.

Findings indicate significant differences between the LMRglc of the utricle and the saccule when compared to the ampullae of the superior, posterior, and lateral semicircular canals. When the LMRglc values for the individual end organs are compared to structures of the central nervous system, the utricle and saccule have values similar to the dorsal horn of the lumbar spinal cord[13] or the hypoglossal nucleus,[14] while the remaining end organs have values lower than those of white matter structures.[8]

Several possible explanations exist for the differences seen in the vestibular LMRglc. One explanation is the distribution of dark cells. It is well accepted that dark cells, via sodium, potassium adenosinetriphosphatase ($Na^+,K^+$-ATPase)[15,16] move ions against a high electrical and concentration gradient. On the basis of their distinct distribution[17] and their high energy requirements, one would expect to find a high LMRglc in regions where the distribution of dark cells is greatest. Therefore, it was expected that the utricle would be the highest, the canal crista would be similar (similar dark cell surface areas[18]), and the saccule would have the lowest LMRglc. This is not the case. Sampling variance could possibly explain some of these differences. A strip of dark cells on the anterior utricular wall that extends from the common crus and into the posterior canal ampulla may have been included in the posterior canal specimen. This would decrease the rate for the utricle while elevating that of the posterior canal. These explanations would not apply to the saccule, since it does not contain dark cells, and it is unlikely that those from the utricle were included in the sample. Here part of the explanation may lie in the reports of this end organ's response to acoustic stimulation,[19] which would increase neural activity and thus LMRglc.

Other possible explanations for these differences in LMRglc may be differences in spontaneous activity, in density of innervation, or in the number of hair cells. However, data from Baird et al[20] and Goldberg et al[21,22] on the vestibular nerve spontaneous activity indicate that while there are differences in regularly and irregularly firing afferents, the total spontaneous activity of the individual end organs is likely similar. Also, there is little difference in the number of nerve fibers[23] or hair cells in the different semicircular canals.[24] The LMRglc differences may instead be related to differences in the ratios of sensory to nonsensory cells. These data are not presently available. There are, however, data for several species dealing with the number of hair cells and the surface area of the end organs.[24] Ratios of these data (number of hair cells per square micron) show that in the majority of cases, the ratio is the smallest in the end organs that have the highest LMRglc. This is, of course, an indirect measure, since it gives no information as to the actual volume of the cells or their metabolic activity.

These differences might also be explained by the anesthesia and/or delivery of the isotope to the inner ear. These explanations are unlikely, given the length of time after anesthesia (>2.5 hours), the other elements of the experimental design, and the fact that inner ear blood flow is autoregulated[25] and therefore isotope delivery to these tissues should be similar from animal to animal regardless of systemic blood pressure. The most likely explanation may simply be that these end organs were stimulated more than the others by "normal" movements during the experiment. This is possible, since the animals were free to move their upper torsos. Some of the animals were actively grooming and exploring their environment, while others slept during part of the experiment. This would also help explain some of the variance seen in the data. Future experiments in anesthetized animals should provide an answer to this point.

In most of the central nervous system, LMRglc and regional blood flow are directly linked.[26] That is, if regional blood flow is high, then LMRglc is also high, and this coupling is maintained during increased activity. While there have been some studies correlating blood flow and glucose utilization in the cochlea following acoustic stimulation,[2] evaluations of regional vestibular blood flow are very limited. Studies on utricular and posterior semicircular canal blood flow and capillary morphology from Lyon et al[27-29] indicate that the capillary length per unit volume is greater in the utricular macula compared to the posterior semicircular canal. This would suggest a greater peak metabolic requirement for the utricle, and our data support this conclusion, on the basis of LMRglc in the "unstimulated" animal.

This does bring up some interesting questions with regard to vestibular blood flow and metabolism. If it is assumed that a cubic millimeter of vestibular end organ is equivalent to a milligram wet weight, then blood flow to the posterior canal ampulla is approximately 4 mL/g per minute, or twice that to the central nucleus of the inferior colliculus (2 mL/g per minute[12]). However, the LMRglc in the vestibular end organs is only 17% that of the inferior colliculus. Blood flow in the vestibular system greatly exceeds the need for delivery of glucose. Data from Kambayashi et al[30] show that the cochlear potential lasts for long periods even when there is no glucose supplied by the circulation. These data suggest that delivery of metabolites is not a primary regulating factor for blood flow. Perhaps in this case the high blood flow rate is for the removal of waste products or the maintenance of ion concentrations, pH, and/or temperature. There are data showing the need to maintain pH levels. Wangemann et al[31,32] have shown that a pH change can alter membrane potential and ion conductance of transitional cells and transepithelial potassium ion transport of dark cells. These are, of course, speculations, and more experimental data are needed before any conclusions can be reached.

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ACKNOWLEDGMENTS — We thank Drs Louis Sokoloff and Carolyn Beebe Smith, National Institute of Mental Health, for their guidance and the use of their equipment.

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*Ann Otol Rhinol Laryngol* 106:1997

# MORBIDITY OF COMBINED THERAPY FOR THE TREATMENT OF SUPRAGLOTTIC CARCINOMA: SUPRAGLOTTIC LARYNGECTOMY AND RADIOTHERAPY

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Many surgeons find that patients have increased morbidity after supraglottic laryngectomy if postoperative irradiation is given, but this remains poorly documented. Twenty-nine patients undergoing supraglottic laryngectomy were retrospectively reviewed. Seventeen received postoperative radiotherapy, and 12 did not. The mean follow-up was 64 months. When compared to patients treated with supraglottic laryngectomy alone, irradiated patients had a significantly higher incidence of lifelong gastrostomy dependency (35% versus 0%; $p = .03$) and acute upper airway obstruction (29% versus 0%; $p = .05$). There was a trend toward greater tracheotomy dependency (24% versus 0%), aspiration pneumonia (35% versus 9%), and delayed independent swallowing (34.8 weeks versus 7.8 weeks) in the patients treated with combined therapy, but this difference was not significant. A morbidity index score was developed to evaluate the overall lifelong morbidity of these patients, and this was found to be significantly higher in patients treated with surgery and radiotherapy (2.29 versus 0.83; $p = .04$). Overall survival was equal in both groups. We have shown that radiotherapy increases the morbidity of supraglottic laryngectomy. This should be considered when planning treatment for patients with supraglottic carcinoma.

KEY WORDS — airway obstruction, decannulation, gastrostomy, morbidity, radiation, supraglottic laryngectomy.

## INTRODUCTION

Conservation surgery for cancer of the supraglottic larynx was first described by Alonso[1] in 1947. The procedure, known as supraglottic laryngectomy (SGL), was formalized by Ogura[2] in his landmark article of 1958. The procedure has changed little since then, and has become accepted as an effective treatment for selected cases of supraglottic carcinoma. The complications of SGL are well known and include aspiration pneumonia, tracheotomy dependency, inability to tolerate oral feedings, upper airway obstruction, and even death. Patients need to be carefully selected for SGL to ensure that they can tolerate the intermittent aspiration inherent in the postoperative course. Careful selection will eliminate some patients with cancers that are anatomically suitable for SGL but who have poor underlying pulmonary function. Murray[3] noted that 19.4% of his large series of SGL patients died of aspiration pneumonia. Both Robbins et al[4] and Bocca et al[5] noted a significant incidence of upper airway obstruction after this procedure (5.8% and 4.9%, respectively).

External beam radiotherapy (XRT) is also frequently used for the treatment of supraglottic carcinoma, either as a sole modality of treatment or in combination with surgery. Radiotherapy is also known to have its own specific complications, ie, xerostomia, dysphagia, hoarseness, excessively thick mucus secretion, chondronecrosis of the larynx, transverse myelitis, and laryngeal edema with airway obstruction, often requiring a tracheotomy. The incidence of severe complications of XRT with supraglottic carcinoma ranges from 6% to 19.2% of patients.[6,7]

One might assume that the combination of SGL and XRT would have at least partially additive effects in terms of complications. However, this is poorly documented in the literature. The purpose of this study was to compare the morbidity of patients treated with SGL and XRT to those treated by SGL alone, and to determine if there is a significant difference between these two groups.

## PATIENTS AND PROCEDURES

A retrospective review was performed for all patients treated for laryngeal cancer by the Division of Otolaryngology–Head and Neck Surgery at the Albany Medical College and the Samuel S. Stratton VA Medical Center (Albany) between 1980 and 1990. Two hundred five patients were identified, of whom 34 underwent SGL. One patient died of an acute myocardial infarction 1 month after surgery and was excluded from this review. Four additional patients

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Presented at the meeting of the American Laryngological, Rhinological & Otological Society, Los Angeles, California, April 19-21, 1993.

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TABLE 1. CLINICAL STAGE

| | *Surgery and Radiotherapy* | | *Surgery Alone* | |
|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* |
| T1 | 0 | 0 | 1 | 8 |
| T2 | 12 | 70 | 11 | 92 |
| T3 | 4 | 24 | 0 | 0 |
| T4 | 1 | 6 | 0 | 0 |
| N0 | 5 | 29 | 9 | 75 |
| N1 | 8 | 47 | 3 | 25 |
| N2 | 4 | 24 | 0 | 0 |
| I | 0 | 0 | 0 | 0 |
| II | 4 | 24 | 9 | 75 |
| III | 8 | 47 | 3 | 25 |
| IV | 5 | 29 | 0 | 0 |

were lost to follow-up shortly after completion of treatment and were also excluded (2 patients had surgery alone and 2 patients had surgery and XRT). The remaining 29 patients form the basis of the study.

There were 25 men and 4 women. The mean age at diagnosis was 57.8 years, with a range of 42 to 71 years. The duration of follow-up was at least 30 months or until death, ranging from 6 to 124 months, with a mean of 64.0 months. All patients underwent standard SGLs as described by Ogura,[2] Bocca et al,[5] and others. A cricopharyngeal myotomy was routinely performed. Seventeen patients (surgery + XRT group) were treated with postoperative XRT with a mean total dosage of 5,770 cGy (range 5,000 to 6,600 cGy). Twelve patients (surgery-alone group) were treated with SGL alone without the addition of XRT.

Patients had been selected for XRT in an uncontrolled manner based on advanced stage of the primary tumor (T3 or T4), presence of nodal metastasis, prophylactic treatment of micrometastasis in lieu of neck dissection, or the discretion of the surgeon. Radiotherapy was delivered to patients receiving postoperative XRT in the following manner. Twelve patients were treated with right and left lateral opposed portals by means of a shrinking field technique, of which 11 also received a direct applied anterior-posterior (AP) field to the supraclavicular area. The average treatment volume was 111 $cm^2$ for the lateral portals (range 45 to 165 $cm^2$) and 120 $cm^2$ for the AP portal (range 90 to 147 $cm^2$). The average initial dosage to the entire area of treatment was 4,510 cGy (range 4,000 to 5,000 cGy). The tumor site was then boosted via bilateral opposed shrunken fields for an additional average 1,478 cGy (range 1,020 to 2,000 cGy) for a total average maximum dosage of 5,930 cGy (range 5,040 to 6,600 cGy). Radiation was delivered via a linear accelerator by means of 6-MeV photons in 10 patients, 4-MeV photons in 1 patient, and cobalt 60 in 1 patient. Seven patients were treated in 200-cGy fractions, and the other 5 in 180-cGy fractions. Finally, 4 of these 12 patients were also boosted with 6-MeV or 9-MeV electrons to the posterior triangle.

The remaining 5 surgery + XRT patients were treated with a single AP portal via a linear accelerator and 4-MeV photons in 200-cGy fractions. The average treatment area was 288 $cm^2$ (range 174 to 360 $cm^2$). The total mean dosage was 5,160 cGy (range 5,000 to 5,800 cGy).

The average time between surgery and initiation of XRT was 55.3 days (range 26 to 87 days). All but 1 patient completed XRT in 29 to 60 days (mean 44.4 days), and the average time from surgery to completion of XRT was 99.7 days (range 56 to 187 days). One patient was noncompliant with the XRT protocol, and although he eventually received a full course of treatment, it took 187 days to complete from the date of his SGL.

Mean age and duration of follow-up were similar in both groups: 57.5 years and 52.3 months, respectively, for the surgery + XRT group, and 58.2 years and 77.6 months, respectively, for the group treated with surgery alone.

Eighty-eight percent of patients in the surgery + XRT group were treated with concurrent neck dissection, and 26.6% of these patients underwent bilateral dissection. A similar rate was seen in surgery-alone patients (82%), and these were bilateral in 30.0% of these patients.

Ten of 11 surgery + XRT patients treated with unilateral neck dissection underwent complete neck dissections, ie, radical neck dissection or modified radical neck dissection with preservation of the spinal accessory nerve (cranial nerve XI). One patient underwent a lateral neck dissection with preservation of the sternocleidomastoid muscle, internal jugular vein, and cranial nerve XI (selective neck dissection). Three of 4 surgery + XRT patients treated with bilateral neck dissection underwent simultaneous bilateral selective neck dissection. The fourth patient was treated with an ipsilateral complete neck dissection and simultaneous contralateral selective neck dissection.

Seven surgery-alone patients were treated with unilateral complete neck dissection. Three additional patients underwent simultaneous bilateral neck dissection, of which 2 were treated with ipsilateral complete neck dissection and contralateral selective neck dissection, and 1 with bilateral selective neck dissection.

Clinical stage at presentation as defined by AJCC criteria (1989 update) is listed in Table 1. Surgery +

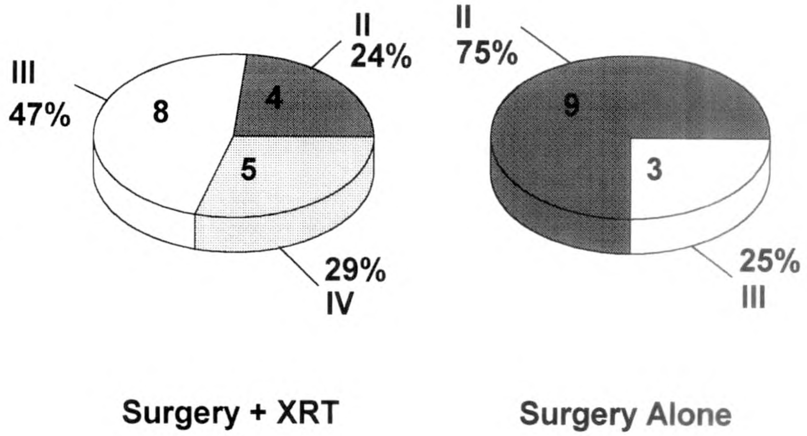

Fig 1. Distribution of clinical staging groups according to treatment. XRT — radiotherapy.

XRT patients presented on average with a higher clinical stage than patients treated with surgery alone. Of the patients with surgery + XRT, stage II was found in 4 of 17 (24%), stage III in 8 of 17 (47%), and stage IV in 5 of 17 (29%). Of the patients with surgery alone, stage II was found in 9 of 12 (75%), and stage III in 3 of 12 (25%; Fig 1).

The morbidity of treatment was compared between patients receiving surgery + XRT and those receiving surgery alone. Morbidity was defined as delayed tracheal decannulation or delayed development of full oral feeding of more than 3 weeks' duration after surgery, or any episodes of aspiration pneumonia, airway obstruction requiring a visit to the hospital, placement of a permanent feeding gastrostomy for inability to tolerate full oral intake, or permanent tracheotomy.

A "morbidity index score" was calculated for each patient by adding the total number of morbid events, as described above, that each patient developed from the time of surgery until death or the end of the study. The average morbidity index score was determined for each group, and the statistical significance of this difference was determined with the Mann-Whitney rank sum test. The incidence of each type of morbidity was also calculated for both groups, and the statistical significance of the difference, if any, was determined with Fisher's exact test.

TABLE 2. HISTOLOGIC STAGE

| | *Surgery and Radiotherapy* | | *Surgery Alone* | |
|---|---|---|---|---|
| | *No.* | *%* | *No.* | *%* |
| T1 | 0 | 0 | 1 | 8 |
| T2 | 11 | 64 | 11 | 92 |
| T3 | 3 | 18 | 0 | 0 |
| T4 | 3 | 18 | 0 | 0 |
| N0 | 3 | 18 | 9 | 75 |
| N1 | 3 | 18 | 1 | 8 |
| N2 | 9 | 53 | 0 | 0 |
| ND | 2 | 11 | 2 | 17 |

ND — no neck dissection performed.

## RESULTS

Clinical stage correlated with pathologic stage in 8 of 10 surgery-alone patients, but only 8 of 15 surgery + XRT patients. Two patients in each group did not undergo neck dissection, and therefore histologic stage is indeterminate. The only variation in the surgery-alone group was 2 patients clinically overstaged for nodal disease. Both patients were initially staged N1, but on final pathology were found to be N0. There were numerous instances of both overstaging and understaging of the primary tumor and nodal disease in the surgery + XRT group. One patient demonstrated evidence of preepiglottic space involvement on preoperative CT (stage T3), which was not confirmed histologically (stage T2). Two patients clinically staged as T2 were found to have histologic evidence of thyroid cartilage involvement (stage T4). Nodal disease was clinically understaged in 5 patients. One N0 patient and 4 N1 patients were found to have multiple involved lymph nodes (stage N2). Finally, 1 patient clinically staged as N1 was histologically N0 (Table 2).

Overall survival for each group was identical: 76% for surgery + XRT and 75% for surgery alone. There was no significant difference in the 2- and 5-year survival between groups: 88% and 60% for surgery + XRT, and 100% and 67% for surgery alone, respectively (Fig 2). There were no local recurrences in patients given XRT. Only 1 patient treated with surgery alone developed a local recurrence, and this patient was salvaged with a total laryngectomy. One patient in each group developed distant metastasis to the lungs. One patient treated with surgery + XRT developed a metachronous primary in the lung 12 months after treatment, as well as a third primary in the parotid gland $5^1/2$ years later. This patient is now alive with disease 89 months after initial surgery.

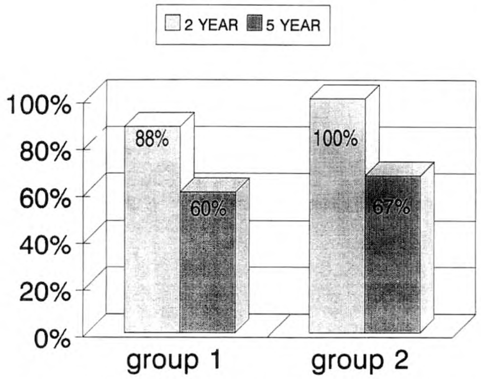

Fig 2. Two-year and 5-year actuarial survival. Group 1 — supraglottic laryngectomy with postoperative radiotherapy, group 2 — supraglottic laryngectomy alone.

Three patients in the surgery-alone group developed second primaries at 36 months (tonsil), 48 months (pyriform sinus), and 50 months (pharynx). The patient who developed a second primary in the tonsil was initially salvaged, but went on to develop third and fourth primary lesions. He finally succumbed to widely disseminated carcinoma 67 months after initial treatment.

Five patients (29.4%) in the surgery + XRT group manifested regional metastases after treatment. This was on the side of a previous neck dissection in 3 patients, and on the undissected side in the other 2. Three of these 5 patients were salvaged surgically. Two patients (16.7%) treated with surgery alone manifested regional recurrence, 1 each in the previously dissected and undissected sides of the neck. One of these patients was salvaged with surgery and XRT (Fig 3).

The overall incidence of recurrence of clinically evident malignancy in the surgery + XRT group was 41% (7 of 17). Three of these 7 patients were salvaged and are now without evidence of disease. Final control in this group was therefore 76.6% (13 of 17). Seven of 12 patients in the surgery-alone group had recurrences (58%). Four of these patients were salvaged and are now without recurrent disease for an overall control rate of 75% (9 of 12; Fig 4).

The patients treated with postoperative XRT suffered increased morbidity compared to those treated with surgery alone (Table 2). The average time from surgery to tracheal decannulation was 14.3 weeks for the surgery + XRT group and 6.8 weeks for the surgery-alone group (p = .18). Of the surgery + XRT patients, 62.5% had delayed decannulation of greater than 3 weeks, and 43.7% of patients had delays of over 3 months. Of the surgery-alone patients, 33% had delayed decannulation of more than 3 weeks, but only 16.6% of patients had delays of 3 months or more. These differences, however, were not statistically significant (p = .32 for 3 weeks and .13 for 3 months). Surgery + XRT patients on average took 34.8 weeks to develop adequate oral intake. Surgery-alone patients were able to swallow and sustain themselves orally after only 7.5 weeks (p = .20). Of the surgery + XRT patients, 56.2% had delayed development of full oral intake of more than 3 weeks, with delays of greater than 3 months in 31.2% of patients. The rates of delay in surgery-alone patients were 41.6% and 25.0%, respectively. Again, these differences were not statistically significant (p = .18 for 3 weeks and .52 for 3 months).

Six patients (35%) treated with surgery + XRT required placement of feeding gastrostomies because of inability to tolerate oral feeding. These were permanent in all 6 patients. All surgery-alone patients were able to develop independent oral intake between 2 and 30 weeks, and none required feeding gastrostomies. This difference was highly significant (p = .026). Five surgery + XRT patients (29.4%) presented with a total of 11 episodes of acute upper airway obstruction. Two patients developed full respiratory arrest, which was fatal in 1 patient. This complication was never seen in patients treated with surgery alone. This difference was also statistically significant (p = .05). Five surgery + XRT patients (29.4%) developed a total of 7 episodes of aspiration pneumonia. Only 1 patient treated with surgery alone developed a single episode of aspiration pneumonia (8.3%). This difference did not reach statistical significance (p = .18). Finally, 4 of 17 patients (23.5%)

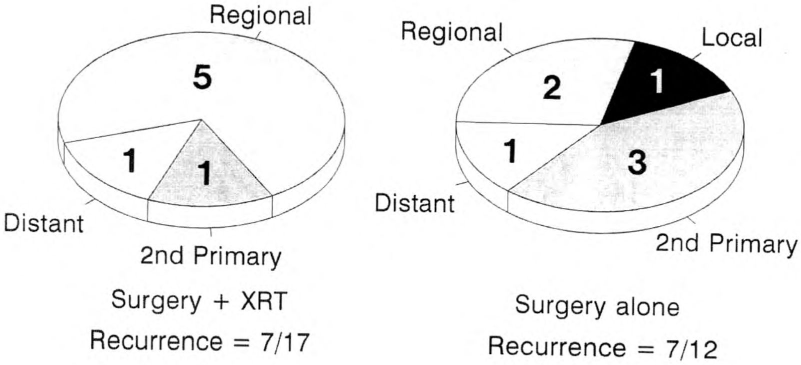

Fig 3. Site of first recurrence of cancer and number of patients that had recurrence at each site. Regional — regional nodal metastasis, local — local recurrence in laryngeal remnant, 2nd primary — second primary cancers at sites outside larynx, or within larynx greater than 36 months after treatment, distant — distant metastasis.

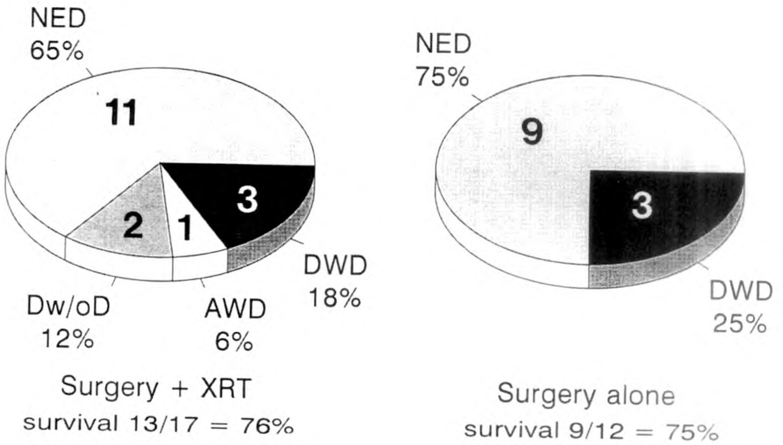

Fig 4. Final outcome of all patients on completion of study or at death. NED — no evidence of disease (alive with no evidence of recurrence), AWD — alive with disease (alive with recurrent carcinoma), DWD — dead with disease (death secondary to recurrent carcinoma), Dw/oD — dead without disease (death of other causes, no recurrent carcinoma).

treated with combined therapy required permanent tracheotomy secondary to upper airway obstruction. In contrast, all but 1 patient treated with surgery alone were decannulated by 18 weeks, and this last patient was decannulated by 32 weeks. No patient in the surgery-alone group required a permanent tracheotomy. However, this was also not statistically significant (p = .10; Table 3).

The morbidity index score is designed to measure the total overall morbidity of the patients treated with each method by combining the above morbid variables into a single score. The mean morbidity index score was 2.29 events per patient for surgery + XRT, and 0.83 for surgery alone (Fig 5). This measure did demonstrate a statistically significant increase in overall morbidity for patients treated with surgery + XRT compared to surgery alone (p = .039).

No difference in morbidity was found within the surgery + XRT group based on the XRT protocol used (bilateral opposed fields versus single AP field). There were insufficient numbers to allow comparison based on total dosage, voltage, or fraction size. In addition, no difference in morbidity was seen based on the type of neck dissection performed.

## DISCUSSION

Supraglottic laryngectomy is an effective procedure for the control of supraglottic cancer in selected patients. Local control rates of up to 100% have been documented by numerous authors.[8,9] External beam irradiation is an accepted addition to the surgical treatment of many head and neck carcinomas, both for the adjuvant treatment of primary lesions, and to try to control the recurrence of regional neck metastases.

Numerous authors have discussed the ability of SGL to control supraglottic cancer, as well as the potential complications of this treatment. However, few authors have explored the difference in morbidity seen in patients treated with SGL and XRT, and compared them to patients treated with surgery alone.

Lee et al[8] noted that 80% of 60 patients treated with SGL were able to resume normal oral intake within 4 months of surgery. They state that the time to oral intake was longer in patients treated with postoperative XRT, but do not document the actual incidence of this delay. They did find that 7 of 50 patients (14%) required prolonged feeding gastrostomies, and that all these patients had received XRT. No patient treated with SGL alone required prolonged use of a feeding tube. Other complications noted were pneumonia 3%, completion laryngectomy for aspiration

TABLE 3. MORBIDITY MEASURED BY DELAY IN DECANNULATION AND ORAL INTAKE

| | *Surgery and Radiotherapy (wk)* | *Surgery Alone (wk)* | *p* |
|---|---|---|---|
| Time to decannulation | 14.3 | 6.8 | .18 |
| Time to oral intake | 34.8 | 7.5 | .20 |

Statistical significance determined with Mann-Whitney rank sum test. Differences between groups are not statistically significant.

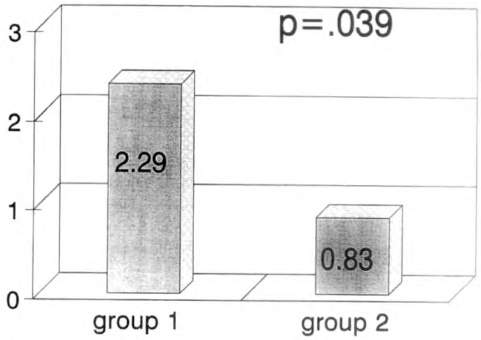

Fig 5. Morbidity index, ie, overall postoperative morbidity measured as mean of total number of morbid events (described in text) per patient for each group. Statistical significance was measured by Mann-Whitney rank sum test p = .039. Group 1 — supraglottic laryngectomy with postoperative radiotherapy, group 2 — supraglottic laryngectomy alone.

TABLE 4. INCIDENCE OF MORBIDITY

| | *Surgery and Radiotherapy (%)* | *Surgery Alone (%)* | *p* |
|---|---|---|---|
| Gastrostomy | 35.0 | 0 | .03 |
| Airway obstruction | 29.4 | 0 | .05 |
| Permanent tracheotomy | 23.5 | 0 | .10 |
| Aspiration pneumonia | 29.4 | 8.3 | .18 |

Statistical significance determined with Fisher's exact test. Differences found to be significant for gastrostomy and airway obstruction only.

5%, permanent tracheotomy 2%, and death 3%, but these complications were not grouped according to treatment modality (surgery alone versus surgery + XRT).

Robbins et al[4] noted that 41% of patients treated with SGL had significant aspiration. Twelve percent of 34 patients in their series treated with both SGL and XRT developed aspiration pneumonia, with 1 patient dying of this complication. The incidence of prolonged feeding gastrostomy and tracheotomy dependence were both 8% for patients treated with combined therapy. These complications were not seen in patients treated with surgery alone. These authors stated that the rate of complications of deglutition was "higher in patients who underwent postoperative radiotherapy, although not excessively so."[4(p426)]

Soo et al[10] noted that all 79 patients treated with SGL had various degrees of aspiration, but only 1 patient developed aspiration pneumonia. The mean time from surgery to decannulation was 15 days. Seven patients (9%) had delayed decannulation of 2 to 8 months, and all but 1 of these patients received postoperative XRT. The incidence of decannulation delay was 28% in patients receiving postoperative radiotherapy and only 2% in those that did not. The length of time from surgery to full oral intake and the rate of gastrostomy tube dependence were not noted.

Our own series shows a difference in lifelong morbidity between patients treated with SGL and postoperative XRT as compared to patients treated with SGL alone. The length of time from surgery to decannulation and from surgery to full oral intake were both increased for patients treated with postoperative XRT: 19.4 weeks and 48.1 weeks, respectively, for surgery + XRT, versus 6.8 weeks and 7.5 weeks, respectively, for surgery alone, although these differences were not statistically significant (p = .18 and .20, respectively). Ten of 17 surgery + XRT patients were successfully decannulated prior to initiating XRT. Two of the remaining 7 were decannulated within 2 weeks of completing XRT and may have been candidates for earlier decannulation. However, 29% (5 of 17) patients treated with postoperative XRT remained tracheotomy-dependent greater than 1 month after completion of XRT. This incidence of decannulation delay is similar to that seen by Soo et al.[10]

Most patients (62.5%) treated with postoperative XRT were still dependent on feeding tubes 3 weeks after surgery, and a large number (43.7%) were still not swallowing independently by 3 months. In contrast, 33% of patients treated with SGL alone were dependent on tube feedings at 3 weeks, and only 16.6% were still not able to tolerate full oral diets at 3 months. Patients treated with surgery + XRT had a significantly increased incidence of other complications compared to those treated with surgery alone: permanent feeding gastrostomy, 35.3% versus 0% (p = .026), and acute upper airway obstruction, 29.4% versus 0% (p = .05). One surgery + XRT patient who had been previously decannulated died of acute upper airway obstruction 3 months after completing XRT. A second patient developed complete respiratory arrest 3 months after XRT and decannulation, but was resuscitated. There was a trend toward increased pneumonia in the surgery + XRT group compared to the surgery-alone group (29.4% versus 8.3%), and one toward lifelong tracheotomy dependence (23.5% versus 0%), but these differences were not statistically significant (p = .18 and .10, respectively).

A morbidity index score was developed in an attempt to quantitate the overall morbidity from the treatments given. Several measures of morbidity were found to have no statistically significant differences between groups when examined individually. However, the difference in the total number of morbid events, ie, the total life experience of the patient in terms of how the treatments negatively affect the patient's life, demonstrates significantly increased morbidity in surgery + XRT compared to surgery alone. The mean morbidity index score of the surgery + XRT group was 2.29 morbid events per patient, compared to 0.83 for surgery alone (p = .039).

There appeared to be a slight trend toward increased upper airway obstruction in patients treated with postoperative XRT if a tracheotomy tube was present during irradiation. Twenty percent of patients (2 of 10) decannulated prior to XRT suffered acute upper airway obstruction later in life, compared to 43% of patients (3 of 7) who were not decannulated until after completion of XRT. However, this difference was not statistically significant. The cause of this trend is unclear. It is possible that the presence of a tracheotomy tube during XRT would predispose the patient to laryngeal or tracheal chondritis leading to obstruction after decannulation, but this is speculative and may only be coincidental. In all but 1 instance, airway obstruction was a result of an inability to handle thick, tenacious secretions, rather than edema of the laryngeal remnant. In addition, there is a poor temporal association between decannulation and the first episode of obstruction in 2 of 3 patients irradiated prior to decannulation (ie, 2 weeks, 3 months, 3 years).

Supraglottic laryngectomy is an effective procedure for the local control of supraglottic carcinoma.[8,9] Regional nodal recurrence is the most common cause of treatment failure.[7,9,11,12] Numerous authors have found regional recurrence rates to be between 10% and 40%.[8,12,13] Most authors recommend treatment to both sides of the neck for supraglottic carcinoma. The choice between neck dissection and XRT of the neck remains controversial, especially in the N0 neck. Clinical nodal staging has been shown to be unreliable, with occult disease between 32% and 35%.[7,12,13] Our own series demonstrates understaging of clinical nodal disease in 22% of patients (5 of 23) treated with neck dissection. Lutz et al[9] found that neck recurrences were almost universally found in unoperated necks, with only 3 of 39 regional recurrences on the side of a previous neck dissection. Ramadan and Allen[13] reviewed 63 N0 patients with surgical extirpation of supraglottic carcinoma with either total laryngectomy or SGL. They found that 14.3% of patients suffered a regional relapse in the face of an elective neck dissection, while patients who did not receive surgical treatment to the neck recurred at a rate of 40.5%. Postoperative XRT did not seem to affect the regional relapse rate. Weber et al[14] recently demonstrated that there is no increased morbidity when performing bilateral neck dissection compared to unilateral neck dissection in patients with supraglottic carcinoma. A significant advantage of bilateral neck dissection is that it allows the accurate determination of histologic nodal stage of both the ipsilateral and contralateral sides of the neck. In light of the previously noted incidence of inaccurate clinical staging, this information is useful when determining the need for additional therapy.

A significant criticism of this study is that the group treated with combined therapy presented with more advanced disease than the group treated with SGL alone, in terms of both primary disease and regional adenopathy (Table 1). Differences in T staging were due to a higher incidence of preepiglottic space and medial pyriform sinus involvement (T3) or laryngeal cartilage erosion (T4) in the surgery + XRT group. In spite of this, local control was identical in both treatment groups, ie, 100% versus 96%.

All patients underwent a standard SGL. Only 1 patient (surgery + XRT) presented with extension of tumor into the vallecula, requiring partial excision of the posterior tongue base. Otherwise, no additional base of tongue resection was required in either group. Eighty-eight percent of surgery + XRT patients and 82% of surgery-alone patients were treated with concurrent neck dissection. These were bilateral in 27% and 30%, respectively. One would therefore not expect different postoperative morbidity in these two groups other than that caused by XRT, since their surgical treatment was nearly identical.

Our study, and others before it, have documented that the regional lymph nodes are the most common site of treatment failure for squamous cell carcinoma of the supraglottic larynx. Some patients may benefit from the addition of postoperative XRT in an attempt to increase the control or prevent the manifestation of regional lymph node disease. However, a heavy price is paid in terms of increased morbidity, as shown in this report. This should be considered when planning the overall treatment package for patients with supraglottic carcinoma. There will be patients who are unsuited to SGL because of a need for XRT and an inability to tolerate the added morbidity described in this report. Such patients should be considered for other treatments (ie, total laryngectomy and XRT, etc).

We therefore now treat our patients at Albany Medical College with supraglottic cancer according to protocols previously described by Bocca et al[15] and others[8,16]: SGL when indicated according to extent of tumor and medical condition of the patient, together with concurrent bilateral neck dissection. A complete neck dissection, preserving the spinal accessory nerve when possible, is performed in the presence of clinically palpable lymphadenopathy. Selective lateral neck dissection, preserving the sternocleidomastoid muscle, internal jugular vein, spinal accessory nerve, and submandibular gland, is performed in the clinically N0 neck. This allows complete histologic staging of all nodal groups at significant risk for metastasis, while minimizing surgical morbidity.[17] Postoperative XRT is reserved for patients with multiple histologically positive lymph nodes, extracapsular spread of nodal metastasis, close surgical margins, or invasion of tumor outside the confines of the larynx. This protocol adequately treats both the primary and the regional nodal disease, while sparing the patient the increased morbidity of postoperative

XRT in many cases.

## CONCLUSIONS

1. Patients treated with SGL have increased morbidity if combined therapy with postoperative XRT is given. Patients treated with both SGL and XRT demonstrated a trend toward delayed decannulation and delayed oral diet, as well as a significantly increased incidence of gastrostomy dependence (p = .026) and airway obstruction (p = .05) when compared to patients treated by surgery alone.

2. Overall lifelong morbidity as measured by the described morbidity index was significantly increased for patients treated with postoperative XRT (p = .039).

3. Regional neck recurrence is the most common cause of treatment failure in this series of patients, as had been previously noted by numerous authors.

4. Bilateral neck dissection allows complete histologic evaluation of regional nodal disease, and may help to determine the need for adjunctive postoperative XRT.

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ACKNOWLEDGMENT — The authors thank Paul L. Feustal, PhD, for the performance of the statistical analysis.

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*Ann Otol Rhinol Laryngol* 106:1997

# MAJOR SURGICAL COMPLICATIONS

# RUPTURED PEXIS AFTER SUPRACRICOID PARTIAL LARYNGECTOMY

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## INTRODUCTION

Supracricoid partial laryngectomies (SCPLs) comprise the cricohyoidoepiglottopexy (CHEP) and the cricohyoidopexy (CHP) procedures. This new concept in partial laryngeal surgery was initially described in the French-language medical literature by Majer and Rieder,[1] Labayle and Bismuth,[2] and Piquet et al.[3] In the 1990s, various reports in the English-language medical literature have addressed the surgical technique, the indications, the oncologic value, and the limitations for SCPL.[4-11] The reported data suggested that SCPL allowed a reduction of the indications for total laryngectomy in advanced endolaryngeal carcinomas, and increased the incidence of local control for early endolaryngeal carcinomas as compared to the classic conservation operations, namely, vertical and horizontal partial laryngectomies.[4-11]

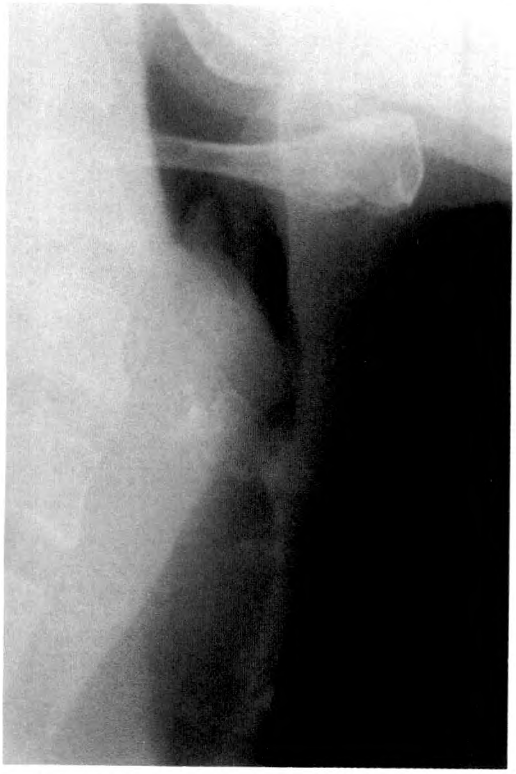

Fig 1. (Case 2) Lateral radiograph of neck demonstrating separation between hyoid bone and cricoid cartilage.

Three hundred seventy-one SCPLs were performed at Laënnec Hospital between 1974 and 1996. Retrospective analysis of the medical charts and operative files revealed that 3 patients had separation between the hyoid bone and the cricoid cartilage resulting in a ruptured pexis postoperatively. A review of the diagnosis, management, and outcome of these 3 cases, as well as an additional case performed by the last

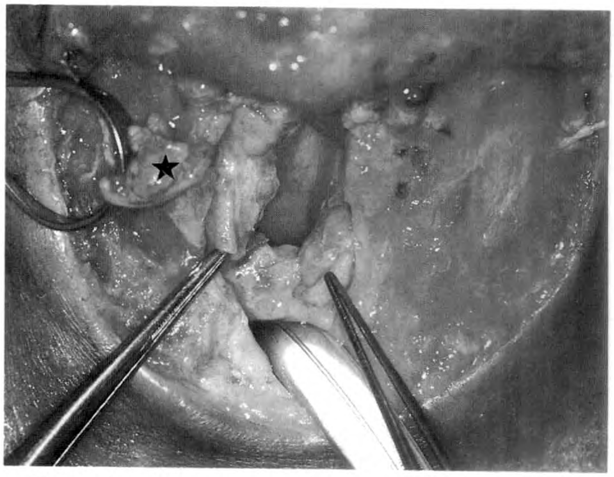

Fig 2. (Case 3) Ruptured pexis (perioperative view) with 3-cm gap between upper border of first tracheal ring and hyoid bone. Star shows transected cricoid arch that will be resected. Instruments point at remaining cricoid edges.

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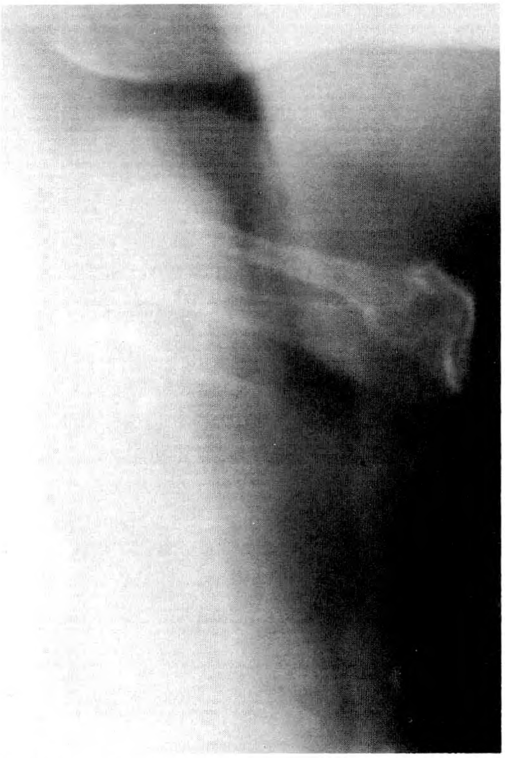

Fig 3. (Case 3) Lateral radiograph of neck demonstrating proper impaction between hyoid bone and cricoid cartilage after revision procedure following ruptured pexis.

author in the United States, is presented and discussed.

## CASE REPORTS

*Case 1.* A 58-year-old man with glottic squamous cell carcinoma (and impaired motion of the true vocal cord) staged as T2N0M0 according to the 1988 American Joint Committee on Cancer (AJCC) classification system[12] underwent SCPL-CHEP sparing one arytenoid cartilage. Pneumonia from aspiration occurred by the 17th postoperative day. Review of the operative file noted 1) complete resection of the ipsilateral arytenoid cartilage and partial resection of the cricoid cartilage on the tumor-bearing side to ensure free mucosal margins of resection and 2) partial rupture of the remaining lateral part of the cricoid ring on the tumor-bearing side at the time of impaction. Palpation of the anterior part of the neck revealed a 5-cm gap between the hyoid bone and the cricoid cartilage. Lateral radiography of the neck confirmed the ruptured pexis. A percutaneous endoscopic gastrostomy was performed, but aspiration did not improve. Completion total laryngectomy was performed, as the patient refused revision of the pexis. The patient is now 24 months from initial surgery, free of disease, and utilizing esophageal speech.

*Case 2.* A 51-year-old man with a glottic squamous cell carcinoma involving both true vocal cords and the anterior commissure, staged as T1bN0M0 according to the 1988 AJCC staging classification system, underwent SCPL-CHEP sparing both arytenoid cartilages. The postoperative course was unremarkable. Pneumonia from aspiration was noted by the 6th postoperative month. Palpation of the anterior neck revealed a 4-cm gap between the hyoid bone and the cricoid cartilage. Lateral radiography of the neck demonstrated a ruptured pexis (Fig 1). Revision of the pexis was performed. A cricopharyngeal myotomy was associated, as palpation of the esophageal inlet at the time of revision surgery noted hypertonia of the cricopharyngeal muscle. The patient is now 18 months from initial surgery, free of disease, and without aspiration.

*Case 3.* A 43-year-old man with invasive glottic squamous cell carcinoma involving both true vocal cords and the anterior commissure, staged as T1bN0M0 according to the 1988 AJCC staging classification system, underwent SCPL-CHEP sparing both

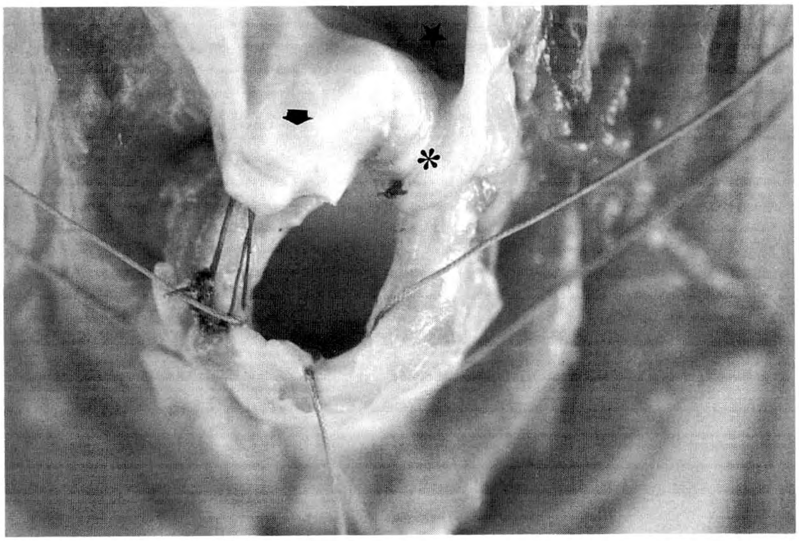

Fig 4. Correct (1 cm apart) suture (Vicryl 1) placement in patient in whom one arytenoid cartilage has been resected. Note that cricoarytenoid region has been covered with spared retroarytenoid mucosa, creating, therefore, bulky mucosal flap (asterisk). Note also 3-0 Vicryl suture between remaining arytenoid cartilage (arrowhead) and upper border of cricoid cartilage, allowing for proper repositioning of remaining arytenoid cartilage. Star — level of posterior pharyngeal wall.

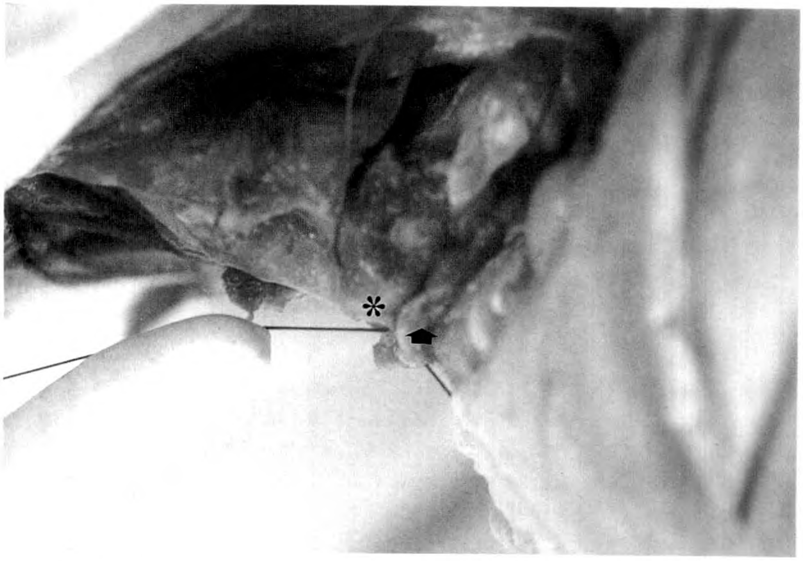

Fig 5. Perioperative view of larynx demonstrating proper impaction between hyoid bone (arrowhead) and cricoid cartilage (asterisk). Note that anterior border of cricoid cartilage and hyoid bone are perfectly aligned, without any gap.

arytenoid cartilages. Removal of the tracheostomy tube was performed by the 6th postoperative day. Massive bleeding through the tracheostoma noted by the 9th postoperative day led to emergency recannulation and surgical hemostasis of an anterior jugular vein. The postoperative course was uneventful, but local examination by the 12th postoperative day noted massive aspiration of saliva, elevation of the skin flap over the tracheostoma, transection of the anterior arch of the cricoid cartilage, and a 3-cm gap between the hyoid bone and the first tracheal ring (Fig 2). Revision of the pexis was performed. Control lateral radiography of the neck demonstrated a correct impaction (Fig 3). The patient is now 6 months from initial surgery, free of disease, and without aspiration.

*Case 4*. A 66-year-old man had two independent squamous cell carcinomas of the larynx. One was a T1 cancer of the petiole of the epiglottis, and the other was a T2 cancer of the true vocal cord according to the 1988 AJCC staging classification system. The patient underwent SCPL-CHP sparing both arytenoid cartilages. The anterior arch of the cricoid cartilage was noted intraoperatively not to be ossified. The patient was noted to have aspiration and elevation of his skin flap above the tracheostomy during the first few days postoperatively. Bedside examination of his larynx revealed a large anterior gap between the cricoid cartilage and the hyoid bone in continuity with a tract beneath the skin flap that led to the tracheostomy site. The patient was returned to the operating room, where it was noted that the sutures had cut through the anterior arch of the cricoid. Revision of the pexis was performed. The patient was readmitted to the hospital 4 months later for a laryngeal stenosis that was managed by laser laryngoscopy. He remained without a tracheostomy for 28 months, and then he was found to have a second primary large cell carcinoma of the lung, to which he succumbed.

## DISCUSSION

Ruptured pexis after SCPL is a rare event. Over a 22-year period of time, the incidence of this complication was 0.8% at Laënnec Hospital (3/371). To our knowledge, this rare complication has been mentioned only once in the literature[13] devoted to SCPL, and no report has been specifically devoted to its management and outcome.

Temporary dysphagia is an expected temporary sequela following SCPL. However, massive aspiration in the immediate postoperative period or chronic aspiration after healing is extremely rare. In such cases, if palpation of the anterior part of the neck demonstrates a gap between the hyoid bone and the cricoid cartilage, then a ruptured pexis should be suspected. Lateral radiography allows for a proper diagnosis; in case 2 it demonstrated a 4- to 5-cm separation between both structures (Fig 1).

Ruptured pexis in our series always resulted in major aspiration, as the pexis is one of the most critical aspects of reconstruction after SCPL. Aspiration was noted in 3 patients before the end of the first postoperative month, suggesting immediate rupture of the pexis due to transection of the cricoid arch by the sutures. This mechanism was documented twice (cases 2 and 4) in our series with bedside visualization of the transected arch of the cricoid cartilage by the Vicryl 1 sutures used at the time of initial surgery. Analysis of the medical charts in the 3 patients who had early ruptured pexis also noted various factors that might have increased the risk for this complication. Partial resection of the cricoid cartilage at the time of initial surgery was noted in case 1. The pexis ruptured a few days following emergency recannulation in an extremely agitated patient (case 3) who was asphyxiating due to massive bleeding at the level of the tracheostoma. A nonossified cricoid was noted at the time of initial surgery in case 4. In the last patient (case 2) ruptured pexis was noted by the 6th postoperative month, suggesting therefore that progressive separation between the cricoid cartilage and the hyoid bone might also have occurred after SCPL.

To avoid a ruptured pexis, tension-free sutures must be placed at the time of initial surgery. This is facilitated by routinely using dissection of the mediastinal trachea to the carina, similar to the technique of resection anastomosis for cervical tracheal stenosis. Experimental data have shown that the primary requirement for the success of tracheal end-to-end anastomosis was the completion of a tension-free anastomotic line.[14] At our departments, as demonstrated in Fig 4, the Vicryl 1 stitches used at the time of pexis are set 1 cm apart from the midline to avoid unnecessary stress on the anterior arch of the cricoid cartilage. In patients in whom the cricoid cartilage is not ossified or the anterior arch of the cricoid cartilage is resected (partially or totally) due to tumor extension, the first two tracheal rings should be looped submucosally. Extreme care to perform a proper impaction as demonstrated in Fig 5 is also mandatory.

In the event of a ruptured pexis we advocate revision of the SCPL with resection of the transected anterior cricoid arch. The new Vicryl 1 sutures are passed around the first two tracheal rings, the hyoid bone, and the tongue base. Release of the epiglottis, if still present, similar to the technique of Kambic et al,[15] allows for bridging of the gap between the tracheal ring and the hyoid bone and proper impaction without unnecessary tension at the level of the suture line. This type of resection and reconstruction, resulting in a tracheocricohyoidoepiglottopexy, has recently been shown not to interfere with the functional outcome.[16] This type of management scheme has allowed for preservation of respiration, phonation, and swallowing without the need for a permanent tracheostomy or gastrostomy in 3 patients in our series. Our data, as well as the 2 successful cases mentioned by Traissac and Verhulst,[13] demonstrate that ruptured pexis after SCPL should not routinely lead to completion total laryngectomy, but rather to a revision procedure.

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*Ann Otol Rhinol Laryngol* 106:1997

# IMAGING CASE STUDY OF THE MONTH

# ODONTOGENIC KERATOCYST IN A YOUNG CHILD

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## INTRODUCTION

The odontogenic keratocyst is a jaw cyst with a proclivity for local invasion and recurrence. The presentation and behavior of this aggressive lesion has been described principally in adults. As the following case illustrates, the odontogenic keratocyst needs to be considered in the differential diagnosis in children who present with mass lesions of the mandible or maxilla as well.

## CASE REPORT

An 8-year-old boy presented with left maxillary and nasal swelling of approximately 1 month's duration. He denied pain, nasal discharge, or visual changes. His parents had noted a gradual change in the alignment of his left maxillary dentition. There was no previous facial trauma or contributory medical history. The family history was negative for basal cell nevus syndrome.

Physical examination (Fig 1) revealed a 4 × 4-cm swelling of the left anterior maxilla and lateral nasal region that extended into the nasal vestibule. On palpation intraorally, the labial and palatal mass was tense and fluctuant. The patient had a dental age of approximately 7 to 8 years, with a class I molar and canine relationship. There was disto-occlusal migration of tooth 9; tooth 10 was unerupted. Cranial nerves V and VII were intact. He did not have diplopia or proptosis. The general physical examination was unremarkable and revealed no dermatologic lesions.

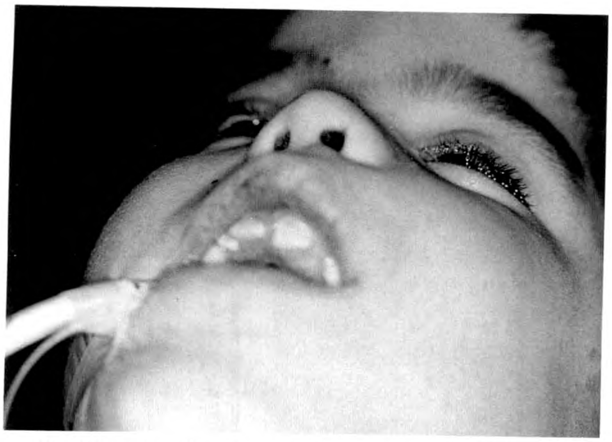

Fig 1. Preoperative photograph demonstrating left nasolabial fullness.

A panorex radiograph (Fig 2) showed migration of the maxillary incisors and canine, as well as resorption of the primary molar roots. A tooth was seen superimposed on the anterior maxillary sinus. Axial and coronal computed tomography (CT) revealed an expansile lesion arising in the alveolar process of the left maxilla (Fig 3). The lesion extended into the hard palate. A plate of bone representing the floor of the maxillary sinus was identified along the superolateral aspect of the lesion, separating the lesion from the sinus. The margin of the abnormality was smooth and sharp, with a thin sclerotic border where the lesion abutted bone. Where the lesion extended beyond the

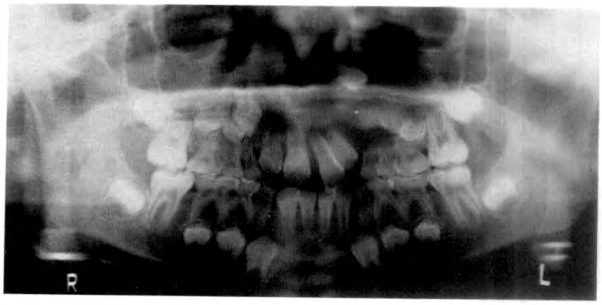

Fig 2. Panorex radiograph demonstrating cystic lesion involving left maxillary sinus, with displacement of several permanent and deciduous teeth.

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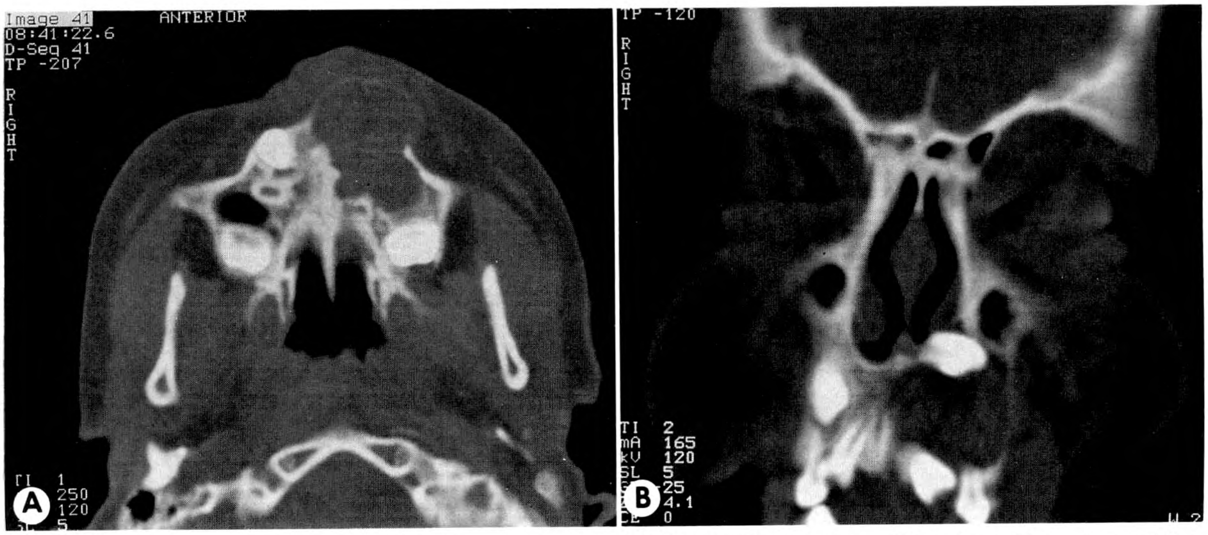

Fig 3. Computed tomograms. A) Axial, demonstrating cystic lesion involving left maxilla and maxillary sinus, with thinning and expansion of anterior maxillary wall. B) Coronal, showing cystic mass in left maxillary sinus, with displacement of multiple teeth. Note permanent left canine tooth displaced superiorly.

maxilla, a thin cortical bony margin remained. The crown of a tooth protruded into the upper part of the abnormality. The inner part of the lesion was homogeneous with a fluid density.

The lesion was surgically removed via a Caldwell-Luc approach (Fig 4). The mass was enucleated and the surrounding bone was curetted. Permanent maxillary canine tooth 11 and primary tooth H were extracted. The maxillary sinus mucosa was removed, a nasoantral window created, and the gingivobuccal incision closed primarily.

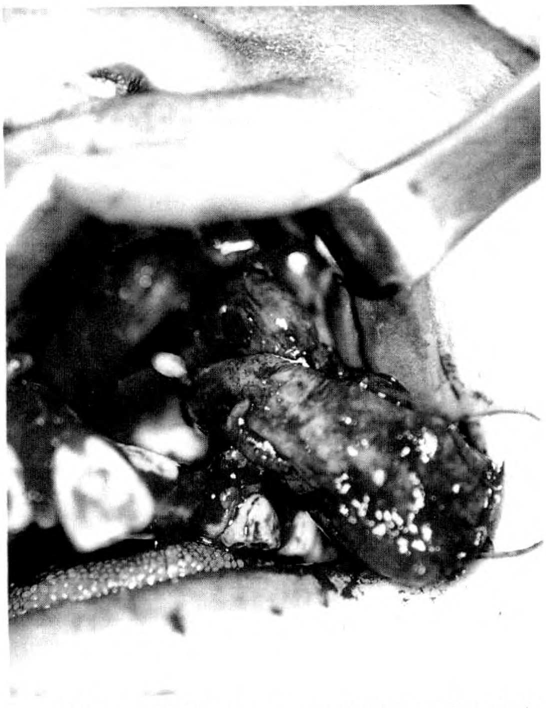

Fig 4. Odontogenic keratocyst completely enucleated via Caldwell-Luc approach.

The diagnosis of odontogenic keratocyst was made based on characteristic histopathology and the associated presence of satellite or daughter cysts (Fig 5).

## DISCUSSION

The odontogenic keratocyst is a developmental odontogenic cyst with a distinctive microscopic appearance. Philipsen[1] first used the term odontogenic keratocyst in 1956 to designate an odontogenic cyst with keratinization of its epithelial lining. A great deal of ambiguity surrounded this lesion until a specific set of defining histopathologic features were delineated.[2] The etiopathogenesis of this lesion was initially assumed to be that of a gingival epithelial hamartoma[3]; most authors now agree that the odontogenic keratocyst originates from the dental lamina.[4,5]

Clinically, the odontogenic keratocyst tends to present in the second or third decade, with perhaps a second peak in the fourth or fifth decade.[4,6,7] Documentation in children less than 10 years of age is rare; the odontogenic keratocyst in this age group is often associated with the basal cell nevus syndrome.[4,6] This autosomal dominant syndrome is characterized by multiple nevoid basal cell carcinomas of the skin and skeletal abnormalities in addition to multiple jaw cysts.

Most studies show a slight male predominance and white racial predominance, with an approximate 2:1 mandibular predominance reported for the site of presentation.[2,4-6] Localized swelling is the typical presentation; spontaneous drainage of cyst fluid is also common; paresthesias, pain, cellulitis, trismus, and nasal obstruction are less frequent initial manifestations.[4,6]

Odontogenic keratocysts are generally unilocular,

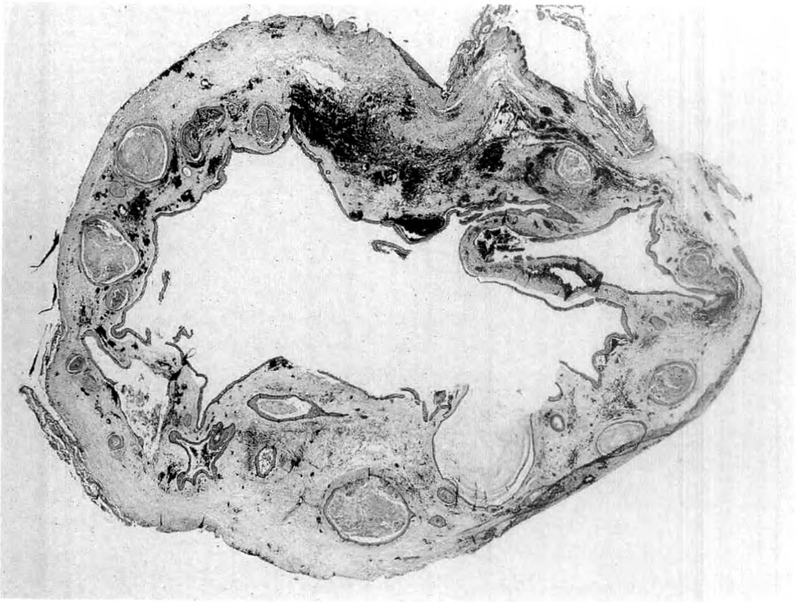

Fig 5. Histopathologic slide demonstrating typical features of odontogenic keratocyst, including 1) epithelial lining, 2) well-defined cuboidal or columnar basal cell layer, 3) cyst wall that is generally thin and uninflamed, and 4) satellite or daughter cysts.

with a thin sclerotic border. Approximately one quarter of these lesions are associated with the crown of an adjacent tooth and 10% to 15% are reported to erode adjacent roots.[4] The radiographic evaluation of cystic jaw lesions has typically included panorex and/or periapical radiographs; the value of CT and magnetic resonance imaging is debated.[7] Preoperative CT does more accurately define the margins of the odontogenic keratocyst, including bone destruction and extension into soft tissue, but the increased radiation exposure may not warrant its routine use.[8,9] A rational approach is to reserve CT scanning for large lesions, particularly those involving the maxilla, where localization of the lesion and extension into the nasal cavity, orbit, or pterygopalatine fossa must be assessed.

A well-circumscribed radiolucent lesion of the mandible or maxilla in a pericoronal relationship is most suggestive of either a dentigerous cyst or an odontogenic keratocyst, but the extensive differential diagnosis also includes such lesions as ameloblastoma, minimally calcifying odontogenic cyst, adenomatoid odontogenic tumor, and ameloblastic fibroma. The preoperative distinction between these lesions is typically not possible by clinicoradiologic means.

The definitive treatment of the odontogenic keratocyst remains uncertain due to the wide variation in reported recurrence rates, ranging from 2.5% to 62.5%.[10] One of two treatment regimens is generally advocated. The conservative approach is a combination of enucleation and curettage.[11] The more aggressive approach includes peripheral ostectomy or en bloc osseous resection with or without cryotherapy or localized chemotherapy with Carnoy's solution.[10] The most aggressive form of treatment, maxillectomy or mandibulectomy with discontinuity, is rarely necessary.

Multiple theories for odontogenic keratocyst "recurrence" have been proposed, including incomplete removal of the original lesion, multiple remnants of dental lamina within the jaws, and satellite cysts.[10,12] Generally, the appropriate follow-up for these patients is a yearly panorex, with the vast majority of recurrences being documented within a 5-year period.[4]

In a 19-year retrospective review at our institution, 11 children were identified with mandibular or maxillary masses of odontogenic keratocyst causation.[13] The age at presentation ranged from 8 to 18 years, with a mean of 13.4 years. A cystic mass with dentition displacement was characteristic radiographically. Treatment principally consisted of enucleation with or without associated extraction of teeth. Follow-up ranged from 1 to 8 years. Seven patients remained disease-free. Recurrences or second primaries occurred in 4 patients, all of whom either had a family history of basal cell nevus syndrome or presented with multiple cysts suggestive of this syndromic diagnosis.

In summary, the clinical behavior of odontogenic keratocysts in the pediatric population appears similar to that reported in adults. Enucleation with extraction of associated dentition is appropriate initial therapy. Recurrences or second primaries should raise the concern of underlying basal cell nevus syndrome, in addition to suggesting the need for more aggressive surgical management. This diagnosis warrants consideration when one is confronted with a child with a mass lesion of the mandible or maxilla.

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ACKNOWLEDGMENT — We thank Robert S. Stern, MD, who shared in the management of this patient.

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*Ann Otol Rhinol Laryngol* 106:1997

## IMAGING CASE STUDY OF THE MONTH

# SONOGRAPHY IN PREAURICULAR PILOMATRIXOMA OF CHILDHOOD

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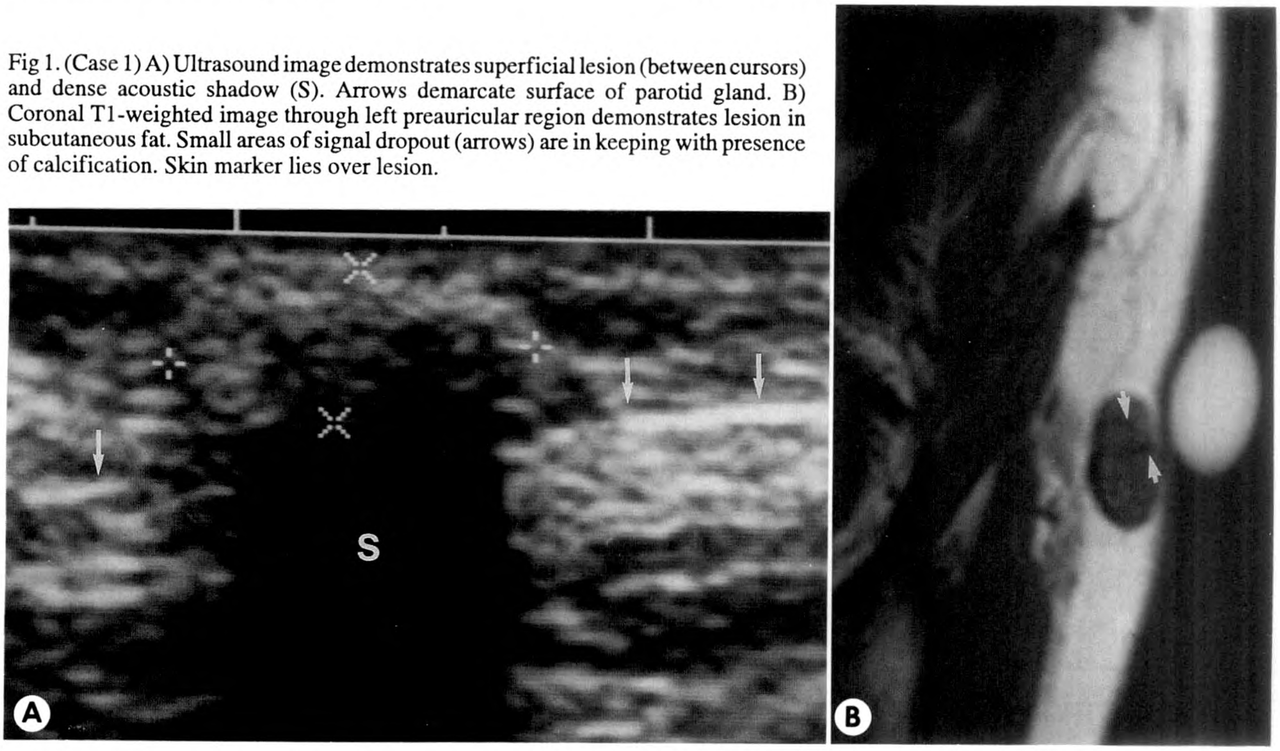
## INTRODUCTION

Pilomatrixomas are one of the most commonly excised superficial masses in children.[1] Most occur in the head and neck region, the preauricular area being a common site. Swellings of the preauricular region may involve the superficial lobe of the parotid gland, in which case surgical excision is associated with the risk of facial nerve injury. Differentiating superficial lesions clinically from those involving the parotid gland can be difficult, and preoperative imaging may be necessary. In young children, imaging by computed tomography (CT) or magnetic resonance imaging (MRI) usually requires general anesthesia. We present two patients with preauricular pilomatrixoma in whom sonography was successfully used to localize the lesion preoperatively.

## CASE REPORTS

*Case 1.* A 3-year-old boy presented with a left preauricular swelling first noted 18 months previously, following trauma to the cheek. It had slowly been increasing in size. On examination the lesion was approximately 1 cm in diameter, superficial, and firm. The overlying skin had a bluish discoloration. He also had a large buttock hemangioma that had been present since birth. Sonography performed using a 7.5-MHz linear array probe demonstrated a well-defined, ovoid, mildly echogenic mass in the subcutaneous fat, superficial to and separate from the parotid gland (Fig 1A). The mass measured 9 × 10 mm in diameter and cast a dense acoustic shadow, in keeping with a calcified lesion. No internal blood flow or feeding artery was demonstrated on Doppler

Fig 1. (Case 1) A) Ultrasound image demonstrates superficial lesion (between cursors) and dense acoustic shadow (S). Arrows demarcate surface of parotid gland. B) Coronal T1-weighted image through left preauricular region demonstrates lesion in subcutaneous fat. Small areas of signal dropout (arrows) are in keeping with presence of calcification. Skin marker lies over lesion.



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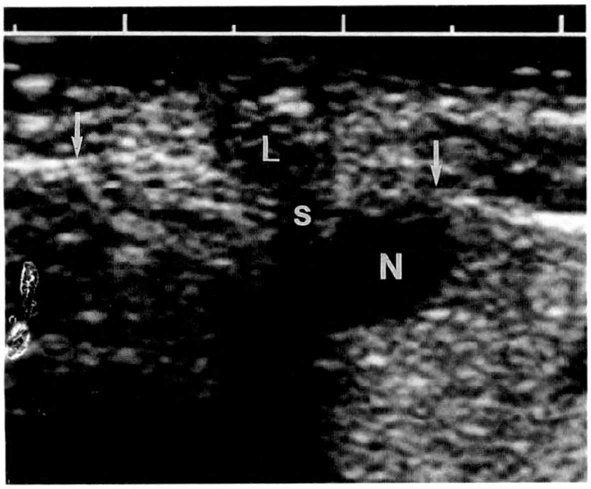

Fig 2. (Case 2) Ultrasound image shows well-defined, very superficial lesion (L) and its acoustic shadow (S). Arrows demarcate surface of parotid gland, which contains hypoechoic lymph nodes (N).

study (not shown here). Because of the possible diagnosis of hemangioma, MRI was performed under general anesthesia. This confirmed the superficial location of the lesion, but there were no features to suggest the presence of internal blood flow or blood degradation products (Fig 1B). At operation, a localized swelling lying immediately lateral to the parotid gland and containing cheesy, gritty material was completely excised. Histologic examination showed sheets of ghost cells with extensive calcification surrounded by fibrous tissue with clusters of foreign body–type giant cells. The features were those of a pilomatrixoma.

*Case 2*. An 11-month-old boy presented with a 5-mm diameter superficial, bluish, firm, smooth swelling in the left preauricular region. It had been present for 2 months and was slowly increasing in size. Sonography using a linear array 7.5-MHz probe demonstrated a 5-mm-diameter spherical mass containing bright echos that cast a dense acoustic shadow and lay in the superficial subcutaneous tissues, separate from the parotid (Fig 2). It had a very different appearance from the lymph nodes noted within the parotid itself, which were of lower echogenicity and did not cast any acoustic shadows. At operation, a superficial subcutaneous mass was completely excised. Histologic examination showed sheets of basaloid epithelium and ghost cells surrounded by connective tissue with many inflammatory cells, including foreign body giant cells. The findings were diagnostic of pilomatrixoma.

## DISCUSSION

Pilomatrixoma (calcifying epithelioma of Malherbe) is a rare, normally asymptomatic subcutaneous tumor that arises from the outer root sheath cell of the hair follicle. Areas of calcification are characteristic, seen in 70% to 80% of cases.[2,3] It is most common during the first two decades of life, but can be seen in all age groups. The head and neck region is the most common site of occurrence, being the location of 67% to 72% of lesions in two recent retrospective studies of histologically proven pilomatrixomas in children.[4,5] Within the head and neck, there is a predilection for the preauricular region.[6,7] The clinical presentation is of a swelling between approximately 0.5 and 5 cm in diameter, the overlying skin having a red or red-blue tinge in 24%.[4] The literature stresses that the clinical diagnosis is difficult,[4,5,7] and that especially in the preauricular area these lesions can be difficult to distinguish from tumors and other soft tissue masses in the superficial lobe of the parotid gland.[6] Other conditions entering into the differential diagnosis are lymphadenopathy (in particular that of mycobacterial infections[3]), hemangiomas, cystic hygromas, preauricular sinuses, and malignant soft tissue tumors such as sarcomas.[4]

Fine-needle aspiration cytology[8] is described as a preoperative diagnostic investigation. This can be very misleading[2] and may at times lead to the erroneous diagnosis of a malignant neoplasm, with potential damaging consequences to the patient.[4]

Previous reports of the use of imaging of pilomatrixomas are limited to the description of the calcification seen on plain radiographs of larger lesions.[9] Parotid sialography has been used in preauricular lesions to demonstrate the medial displacement of the parotid ductal system.[6] The use of sonography has not previously been described. In our hands it has proven extremely useful, demonstrating the well-defined nature of the lesions, their superficial position, and the lack of continuity with the deeper-lying parotid gland, as well as internal calcification, to which it is much more sensitive than plain film radiography. In addition, it is a noninvasive and inexpensive form of imaging, in particular when compared to CT and MRI, and can be performed easily in young children without the need for sedation or general anesthesia.

## CONCLUSION

We have described the use of sonography in the preoperative evaluation of two children with preauricular pilomatrixomas. We have found it a quick, noninvasive, and sensitive investigation. It is reassuring in planning the management of these patients, in whom the clinical presentation can sometimes make estimation of the deep extent of the mass and accurate diagnosis difficult.

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*Ann Otol Rhinol Laryngol* 106:1997

## PATHOLOGY CONSULTATION

# INTRACELLULAR CRYSTALLINE DEPOSITS IN LYMPHOPLASMACELLULAR DISORDERS

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Crystalline immunoglobulin intracellular inclusions may be found in a range of B-cell neoplasias, including MALT (mucosa-associated lymphoid tissue) lymphoma. The crystals are regarded as an outcome of impaired cell secretory activity leading to their accumulation. At times the deposits may be so profound as to obscure the diagnosis and may even lead to misdiagnosis. Examples of the crystals are presented along with a brief literature review.

Intracytoplasmic crystalline deposits have been described in several lymphoplasmacellular disorders, such as plasmacytoma, multiple myeloma, lymphoplasmacytic lymphoma, MALT (mucosa-associated lymphoid tissue) lymphoma, and amyloidosis.[1-4] The crystals may be found within plasma cells, lymphoma cells, or histiocytes, and their formation may be associated with circulating κ light chains or, rarely, with an asymptomatic M component.[2] Immunohistochemical and ultrastructural characteristics of the crystals point to a formation from monoclonal immunoglobulin.[2]

Because the histologic appearance of the crystals can be so unusual and striking and also because they are infrequently encountered, surgical pathologists may encounter difficulty in diagnosis as well as offering judgments on the clinical implications of the deposits. Kapadia et al,[4] in their report of three patients with head and neck (mucosal and lymph node) presentations, underscore these considerations. In each of the three patients, the initial diagnosis was

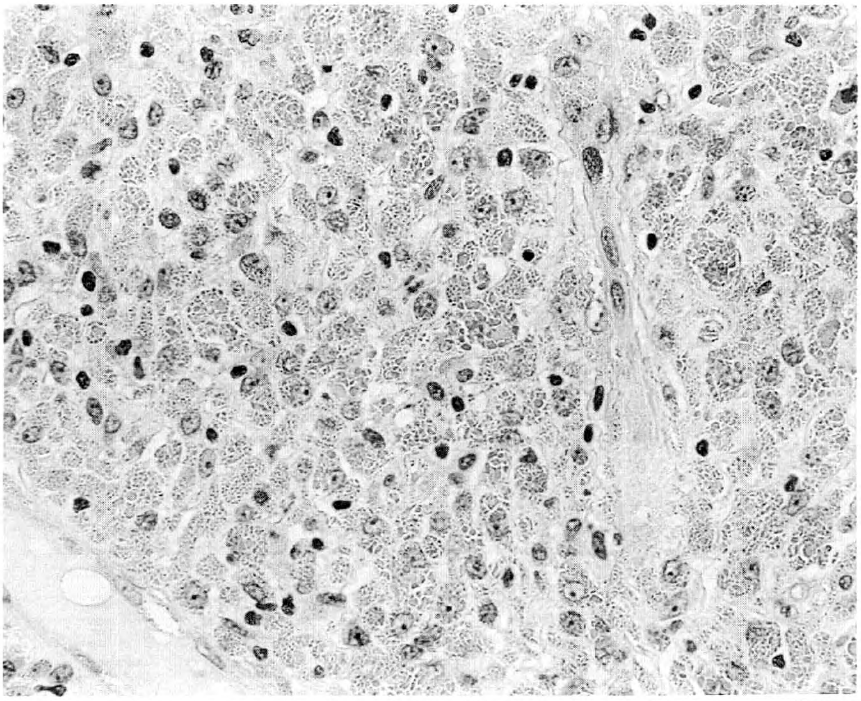

Fig 1. Plasmacytoma of parotid gland in 73-year-old man (H & E, original ×80). Cytoplasm of plasma cells is distended by granular, eosinophilic cytoplasmic inclusions (κ light chain–positive).

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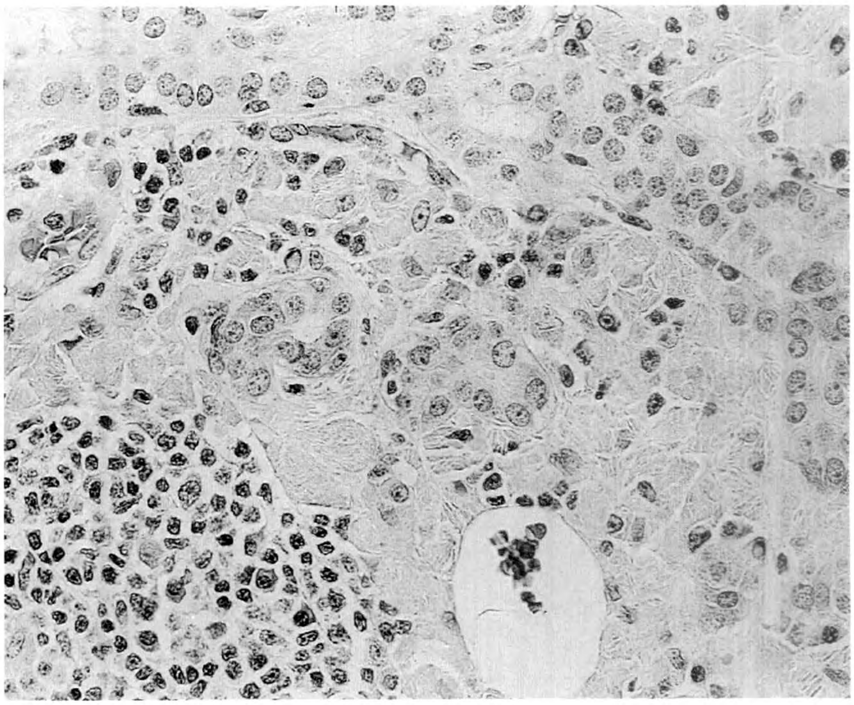

Fig 2. B-cell lymphoma (lymphoplasmacytic) with crystal-storing histiocytosis of palate in 86-year-old woman (H & E, original ×160). Crystals in histiocytes are eosinophilic and irregular in size and shape.

adult rhabdomyoma. Crystal-storing histiocytosis was the eventual final diagnosis. Harada et al[5] published a report of a patient with crystal-storing histiocytosis associated with lymphoplasmacytic lymphoma. In this case, the disease clinically mimicked Weber-Christian disease with prominent head and neck subcutaneous nodules. A different type of crystal was found in the plasmacytoma of the parotid gland reported by El-Naggar et al.[6] Fend et al[7] described an immunoblastic lymphoma of cervical lymph nodes with crystalline inclusions in plasma cells. An accompanying gastric MALT lymphoma had similar inclusions.

In Figs 1-4 we illustrate crystals diagnosed in three patients at the University of Texas M. D. Anderson Cancer Center's Department of Pathology. The plasmacytoma of the parotid gland is similar to that reported by El-Naggar et al.[6] The other two patients had crystal-storing histiocytosis associated with extranodal lymphoplasmacytic lymphomas.

There is probably more than one mechanism re-

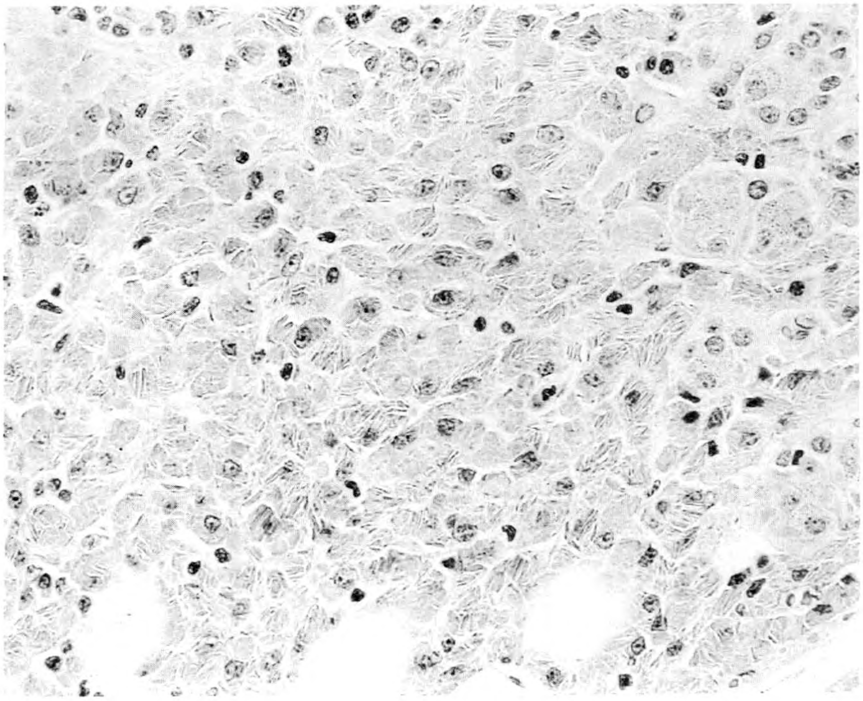

Fig 3. B-cell lymphoma (lymphoplasmacytic) of submandibular lymph node in 60-year-old woman (H & E, original ×100). In this lymphoma, crystal-storing histiocytes have nearly obscured lymphoplasmacytic cells.

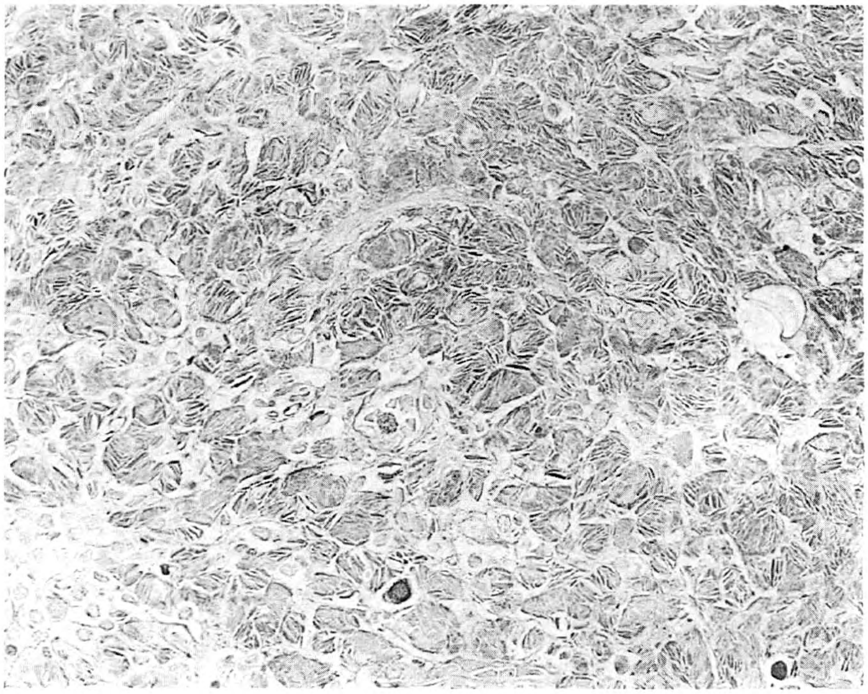

Fig 4. Same patient as in Fig 3. Degree of lymph node and cellular architecture replacement by crystals is clearly seen (H & E, original ×100).

sponsible for the accumulation of the intracytoplasmic crystallized immunoglobulin noted in our patients and in the other reported examples. The variation in size and shape of the crystals speaks to this point. In the cases associated with lymphoplasmacytic lymphomas, they can appear as rodlike structures in rough endoplasmic reticulum or in the perinuclear cisternae.[2] In plasma cell dyscrasias, the crystals may be rectangular or square, finely uniform or globular. In the instances in which immunohistochemical studies have been done, the majority of the crystals can be stained with anti-light and/or anti-heavy chains of immunoglobulins.[2,6] They often manifest a light chain restriction. The crystals seem to contain whole or fragmented portions of immunoglobulin and are different from Russell bodies, which do not show an internal stratification.[2,8]

To date, the crystals have tended to appear in B-cell lymphomas with a prominent plasma cell differentiation. Here, the crystals can be found in histiocytes, which absorb the immunoglobulins, or in the lymphoma cells themselves.

Although similar crystals may occasionally be found in nonlymphomatous plasmacellular lesions, the frequent reporting of their presence in lymphomas may mean they can serve as a marker for these malignancies.

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# CONTENTS

## *INDEPENDENT CONTRIBUTIONS*

**Use of a Composite Fascial Carrier for Laryngotracheal Reconstruction**
Pierre R. Delaere, MD, PhD; Phillip N. Blondeel, MD; Robert Hermans, MD;
Paul J. Guelinckx, MD, PhD; Louw Feenstra, MD, PhD ........ 175

**Migraine-Related Vestibulopathy**
Stephen P. Cass, MD, MPH; Joseph M. Furman, MD, PhD; Jennifer K. P. Ankerstjerne, MD;
Carey Balaban, PhD; Sertac Yetiser, MD; Barlas Aydogan, MD ........ 182

**"Endolymphatic Sacitis" in a Case of Active Meniere's Disease. An Ultrastructural Histopathologic Investigation**
Niklas Danckwardt-Lillieström, MD; Ulla Friberg, MD, PhD;
Anders Kinnefors, MD; Helge Rask-Andersen, MD, PhD ........ 190

**Role of Ultrasound in the Assessment of Vocal Cord Function in Infants and Children**
Ellen M. Friedman, MD ........ 199

**Perceived Hearing Handicap of Patients With Unilateral or Mild Hearing Loss**
Craig W. Newman, PhD; Gary P. Jacobson, PhD;
Gerald A. Hug, MA; Sharon A. Sandridge, PhD ........ 210

**Increase in Expression of IL-4 and IL-5 mRNA in the Nasal Mucosa of Patients With Perennial Allergic Rhinitis During Natural Allergen Exposure**
Chul Hee Lee, MD; Chae-Seo Rhee, MD; Seung Ha Oh, MD;
Yang-Gi Min, MD; Myung Sook Lee, MSc ........ 215

**Distortion Product Emissions in Normal-Hearing and Low-Frequency Hearing Loss Carriers of Genes for Waardenburg's Syndrome**
Xue Zhong Liu, MD, PhD; Valerie E. Newton, MD, MRCP ........ 220

**Salivary Gland Double Tumor: Synchronous Ipsilateral Pleomorphic Adenoma and Acinic Cell Carcinoma of the Parotid Gland**
Ines Misselevich, MD; Ludwig Podoshin, MD;
Milo Fradis, MD; Jochanan H. Boss, MD ........ 226

## *EXPERIMENTAL STUDIES*

**Signal Transduction Pathways in Modulation of Ciliary Beat Frequency by Methacholine**
Bin Yang, MD; Rodney J. Schlosser, MD; Thomas V. McCaffrey, MD, PhD ........ 230

**Effect of Intraoperative Ischemia on Chondrogenesis by Vascularized Auricular Perichondrium Grafted in the Airway**
Saman Naficy, MD; Ramon M. Esclamado, MD ........ 237

**Mucociliary Clearance of Stented Laryngotracheal Tract in Guinea Pigs In Vivo**
Shiann Yann Lee, MD, FICS; Te Huei Yeh, MD; Jiann Chyuan Chen, MD ........ 240

## *MAJOR SURGICAL COMPLICATIONS*

**Posttransplant Lymphoproliferative Disease Presenting as Sudden Respiratory Arrest in a Three-Year-Old Child**
Karin Hague, MD; Peter Catalano, MD; Michael Rothschild, MD; James Strauchen, MD; Billie Fyfe, MD ........ 244

**Prevention of Flash Fires During Facial Surgery Performed Under Local Anesthesia**
Brian K. Howard, MD; Joseph L. Leach, MD ........ 248

## *IMAGING CASE STUDY OF THE MONTH*

**Brown Tumor in the Cricoid Cartilage: An Unusual Manifestation of Primary Hyperparathyroidism**
George Blinder, MD; Nurith Hiller, MD; Netta Gatt, MD; Michal Matas, MD; Shmuel Shilo, MD ........ 252

## *CLINICOPATHOLOGICAL CONSULTATION*

**Yolk Sac Tumors (Endodermal Sinus Tumors) of the Extracranial Head and Neck Regions**
Kenneth O. Devaney, MD; Alfio Ferlito, MD ........ 254

## *MISCELLANEOUS*

**Information for Subscribers** ........ 3a

**Index to Advertisers** ........ 18a

*Ann Otol Rhinol Laryngol* 106:1997

# USE OF A COMPOSITE FASCIAL CARRIER FOR LARYNGOTRACHEAL RECONSTRUCTION

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A vascularized fascia flap may be used as a vascular carrier for free mucosal and cartilage grafts. This composite reconstruction technique has been performed in clinical situations with complex laryngeal or tracheal defects. This paper describes the technique and the rationale for applying this concept to some particular laryngotracheal defects. Morphologic and histologic aspects of this composite reconstruction technique using a transferable recipient bed are discussed.

KEY WORDS — fascia, laryngotracheal reconstruction, vascularized fascia.

## INTRODUCTION

Morphologic repair of parts of the laryngeal or cervical tracheal wall remains a challenge. Reconstruction may be necessary after tumor removal or to achieve a lumen augmentation in cases of airway stenosis. Mostly, the problems of reconstruction can be avoided, such as after segmental tracheal resections, with bridging of the defect by primary closure.[1] In addition, most of the defects resulting after partial vertical laryngectomy require no reconstruction, because the raw surfaces can be left to heal by second intention.[2]

Situations in which regional or distant tissues are needed to preserve the morphologic and functional integrity of the laryngotracheal tract require a complex reconstructive strategy. The ideal tissues for repair of large laryngotracheal defects should have an epithelialized surface without narrowing the lumen, a framework to maintain the airway, and a good blood supply. With the exception of an epiglottis that can be pulled downward for reconstruction of anterior laryngeal defects, tissues with a combination of these requirements are not available in a single donor site.[3]

Cartilage,[4] periosteum,[5] pericardium,[6] and bone[7] have all been used as a monocomponent in laryngotracheal reconstruction. The practical simplicity of these tissues is marred by the fact that the materials used are mesenchymal and, therefore, inevitably produce granulation tissue on their surface exposed to the laryngotracheal air column. Presumably, with time, epithelialization could occur, as well as wound contraction. This results in a reepithelialized but largely contracted wound.[8]

Grafts and flaps containing skin or mucosa allow first-intention healing. Regional musculocutaneous flaps are used to reconstruct anterior laryngotracheal defects.[9] Skin inside the airway has, however, the disadvantages of having a keratin surface that is difficult to "wet," and of transferring hair to the larynx or trachea. Respiratory mucosa can be used for laryngotracheal lining as part of a composite nasal septal graft.[10] Despite the use of ciliated epithelium and autogenous cartilage, the composite septal graft is completely avascular and has to rely on the vascularity of the wound margins for its survival. Furthermore, the graft is limited by size and restricted to the cartilaginous nasal septum.

A possible solution for this reconstructive problem is to address several donor sites and to compose artificially the desired reconstructive tissue. A mucosal graft for internal lining and a cartilage graft for external support can be combined in a composite reconstruction with sufficient blood supply. The vascularization may be provided by a thin connective tissue flap serving as a vascular carrier. The reliability of a vascularized fascia flap used as a transferable bed for mucosa and cartilage in airway reconstruction was first studied in an experimental animal model.[11] It was shown that this flap could bring mucosal and cartilage grafts successfully into an airway defect, preserving the full viability of the grafts without shrinkage of the defect area. This experimental research was translated to the clinical situation with construction of a composite tissue based on a fascial vascular carrier for transplantation of mucosal and cartilage grafts to laryngotracheal defects. Technique,

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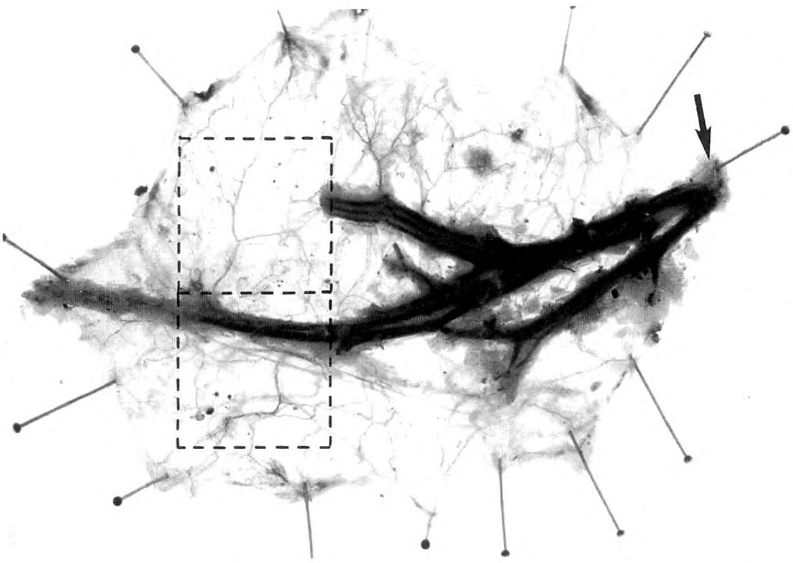


Fig 1. Lateral thoracic fascia flap. Extent of fascial dissection with attached thoracodorsal pedicle (arrow). Branching vascular pattern within tissue is visible to extreme periphery of fascia. Dotted lines — site on which mucosal grafts are sutured to fascia.

possible indications, and postreconstruction morphology will be discussed. The clinical concept is linked with the originally described experimental counterpart in order to discuss the reconstruction technique histologically.

## SURGICAL TECHNIQUE

### CHOICE OF TISSUES

*Vascular Carrier*. A transferable bed useful in laryngotracheal reconstruction should be thin, be potentially available in large amounts, and have a dependable vascular pedicle. Furthermore, the flap should leave a minimal cosmetic and functional defect. A free fascia flap seems most suitable in this respect.

Experimentally, the lateral thoracic fascia flap was used to explore the concept of the composite reconstruction technique. A sheet of fascia situated on the lateral chest wall, measuring 15 × 5 cm, was developed and used in rabbits. This flap is axially perfused by the lateral thoracic vessels.[11]

For clinical use, we also wanted to explore the donor site with the potentially largest amount of fascia available. It has been reported that the thoracodorsal pedicle vascularizes a purely fascia flap overlying the whole serratus anterior muscle.[12] To document the small vessel distribution and the amount of tissue available within the lateral thoracic fascia (serratus fascia), we performed injection studies of the thoracodorsal artery. This was done on fresh human cadavers with injection of the thoracodorsal artery using a 22-gauge catheter. The artery was flushed with normal saline, and then 20 mL of blue Microfil (Canton Bio-Medical Products Inc, Boulder, Colo) was injected into the vessel. One hour after Microfil injection, the skin was elevated and the thoracodorsal artery was dissected distally with the lateral thoracic fascia overlying the serratus anterior muscle. The thoracodorsal arterial branch to the latissimus dorsi muscle was divided. Flaps of 12 × 15 cm could be raised with a fine branching network of vessels within the fascial layer (Fig 1).

The serratus fascia ensures large amounts of tissue with good axial perfusion and a long vascular pedicle — characteristics that are beneficial when it is used as a vascular carrier for mucosal and cartilage grafts.

Elevation of the flap starts with a skin incision in the lateral thoracic area with the patient positioned in a 45°, anterior-oblique position. The lateral edge of the latissimus dorsi muscle is identified. The tissue immediately underlying the latissimus dorsi muscle composes the major portion of the lateral thoracic fascia. The thoracodorsal vascular pedicle is dissected and the latissimus dorsi pedicle is ligated and divided. Elevation of the fascia from the serratus muscle is done after mucosal grafting.

*Mucosal Graft*. As in the experimental setting, full-thickness buccal mucosal grafts were chosen for internal lining. Sections of mucosa as large as 3 × 5 cm may be removed from the left and right buccal areas. The mucosa should be taken anterior to the parotid duct opening. To avoid restriction of mouth-opening, the donor defect is closed with a full-thickness skin graft. The mucosal graft is made thinner by trimming the undersurface with scissors.

In order to prevent interference with the revascularization of the graft, two conditions must be met. First, the contact between the graft and the fascial flap must be as intimate as possible and there must be no interposition of blood or serum, which might act as a barrier to the ingrowth of host vessels. Second, satisfactory fixation and immobilization must be pro-

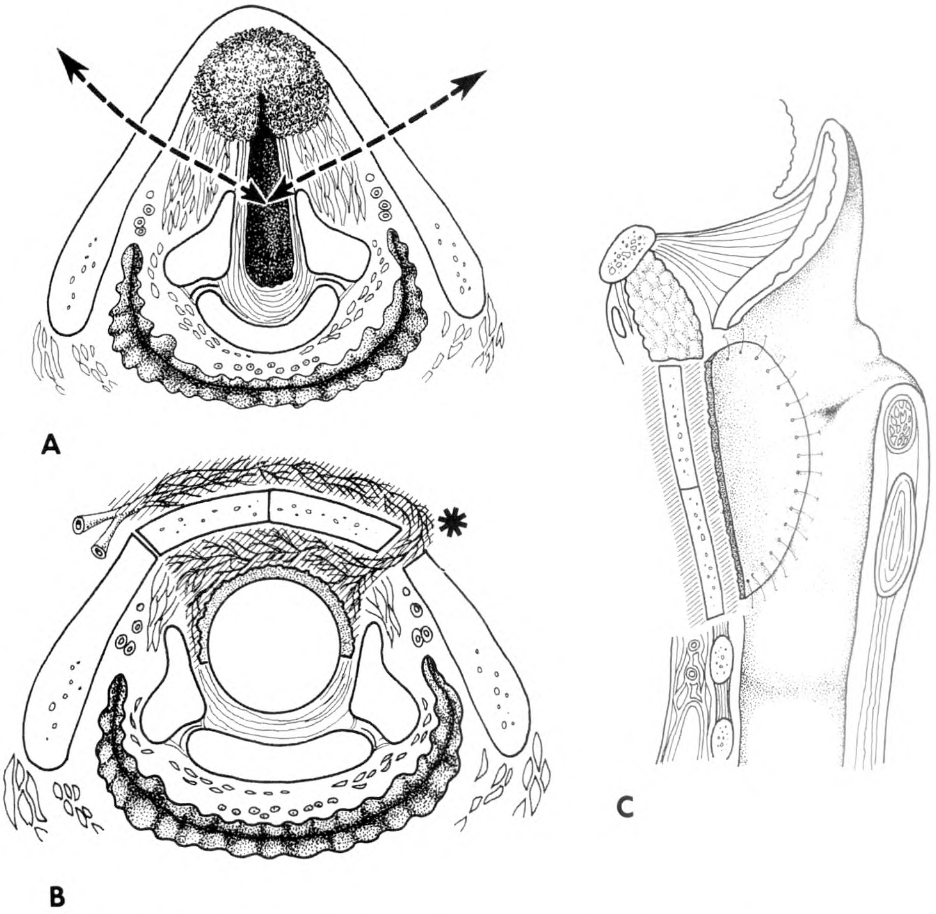


Fig. 2. Reconstruction technique with composite vascular carrier. A) Anterior commissure tumor with subglottic extension. Axial view of tumor extension and resection margins. Dashed lines — extent of surgical resection. B) Axial view of composite reconstruction. Vascularized fascia is 180° rotated (asterisk) to bring mucosal lining inside and to protect cartilage autograft circumferentially in its vascularized bed. C) Sagittal view of anterior laryngotracheal defect reconstructed with composite technique.

vided so that vessels are not torn during the period of vessel penetration into the graft. These two conditions are fulfilled by careful suturing of the graft to the fascia and by leaving a soft silicone stent in place 1 week to keep the graft in contact with the recipient site in the airway.

The mucosal grafts are sutured to the fascia flap with the fascia still attached to the underlying serratus muscle. With several Vicryl 5-0 stitches, the edges of the graft are sutured to the distal end of the stretched serratus fascia (Fig 1). A mucosal-lined fascial area measuring 6 × 5 cm may be obtained. The internal lining provided by this area can match most of the laryngotracheal defects.

*External Support*. Rib cartilage provides a major cartilage transplant source in humans. Experimentally, auricular cartilage was used because in rabbits, the outer ear is more accessible.[11] Clinically, the donor site is taken low on the rib cage, and graft material can be obtained from the distal ends of the 8th, 9th, and 10th ribs. A wide variety of graft sizes, applicable to most reconstructive needs, is available.

### COMBINATION OF LINING, SUPPORT, AND VASCULARIZATION FOR LARYNGOTRACHEAL DEFECT RECONSTRUCTION

A possible indication for this composite reconstruction technique is a laryngotracheal defect that is difficult to repair with reliable local or regional tissues, such as some extended partial laryngectomy defects. The limited descent of the epiglottis may, for example, prevent reconstruction of laryngeal defects extending toward the upper trachea. The composite reconstruction technique may also be useful in augmenting anterior tracheal segments to resolve stenosis when segmental resection with end-to-end reanastomosis cannot be performed. An extended laryngeal defect illustrates how the separate tissues are combined for airway wall reconstruction (Fig 2).

Depending on the internal surface of the defect area to be covered, the mucosal graft may be taken from one or both cheeks. The mucosal grafts are sutured to the superficial fascia still attached to the serratus muscle with Vicryl 5-0. Dissection of the mucosal-lined fascia is then started from inferior to superior, by carefully and sharply dissecting the lateral thoracic fascia of the fibers of the serratus muscle. The long thoracic nerve innervating the serratus anterior muscle might be dissected away from the fascia and left attached to the muscle, thereby avoiding scapular winging. Then, the vascular pedicle is ligated and the entire flap removed from its bed. The lateral thoracic skin is closed over a suction

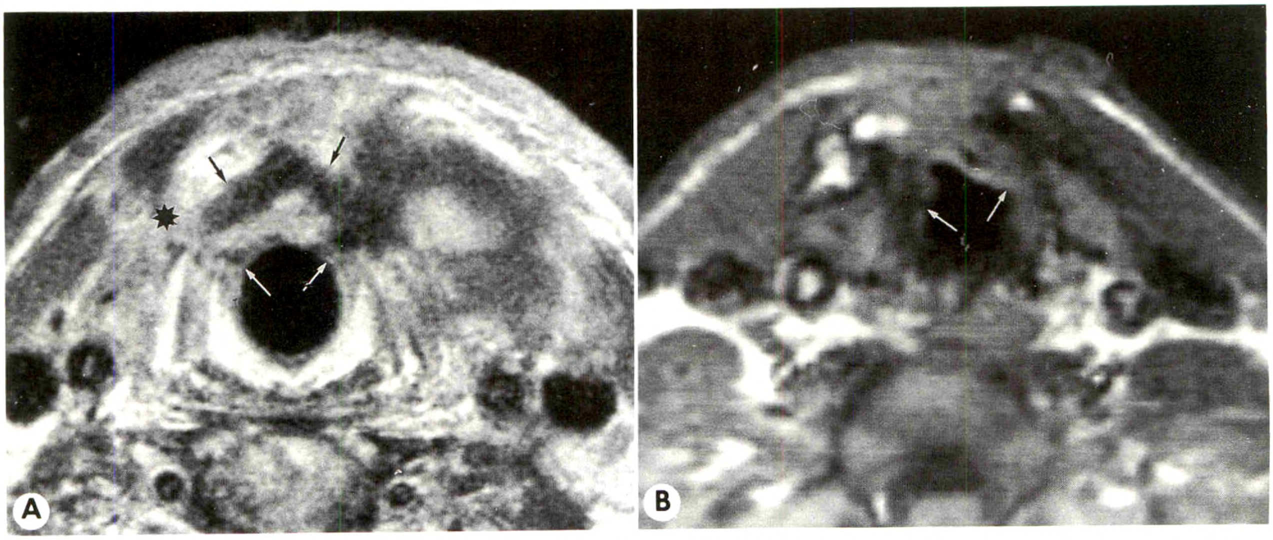


Fig 3. Radiologic evaluation of composite reconstruction technique. A) Gadolinium-enhanced, T1-weighted magnetic resonance image of reconstructed extended partial laryngectomy reveals homogeneous enhancement of fascia flap reconstructing frontal laryngeal defect. Fascia leaves larynx at left side (asterisk) between rib cartilage (black arrows) and thyroid cartilage remnant. Reconstructed internal lining is situated between white arrows. B) Gadolinium-enhanced, T1-weighted magnetic resonance image after anterior tracheal augmentation. Tracheal stenosis was widened by inserting 2-cm-wide patch of revascularized mucosa and cartilage. This was done in patient with history of anastomotic stricture after tracheal resection of 4 cm with end-to-end anastomosis. Restenosis was initially treated with carbon dioxide laser, repeated dilation, and stenting period without success. Mucosal component of composite reconstruction is visible in anterior half of trachea (area between arrows).

drainage system. The mucosal-lined fascia can now be sutured to the mucosa, at the margins of the defect. Suturing is begun caudally. A soft laryngeal silicone stent is put through the defect before placing the final sutures at the upper margin between the buccal mucosa and epiglottis. The stent is placed to obtain some counterpressure, sufficient to maintain contact of the overlying graft with the vascularized fascia bed without exceeding the capillary closing pressure.

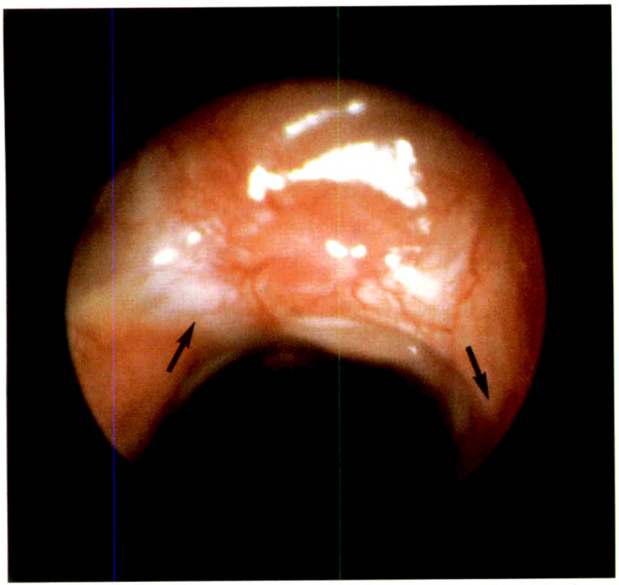


Fig 4. Endoscopy after composite reconstruction. Oral mucosal graft is visible in subglottic area. There is sharp demarcation between stratified epithelium and ciliated epithelium (arrows). Vessels of fascia flap are visible through mucosal lining.

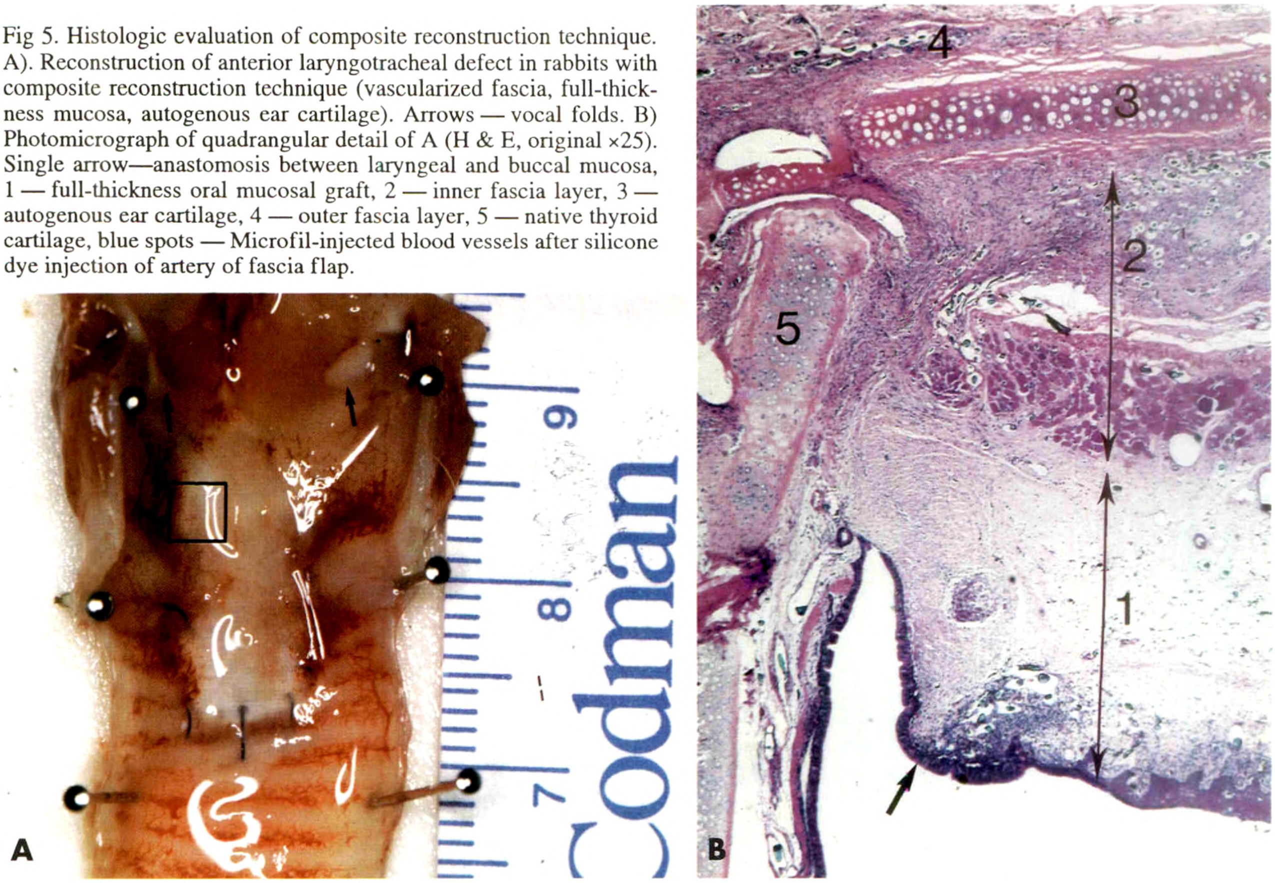
Rib cartilage grafts, taken from ribs 8 and 9, are sculpted and sutured to each other and to the remaining thyroid and cricoid cartilage with 2-0 polypropylene (Prolene) sutures. Perichondrium is left on one side of the graft. The epiglottic remnant is fixed to the upper cartilage grafts (Fig 2C). A small opening between the remaining thyroid cartilage and rib cartilage is left on one side to provide a passage for the vascular carrier. The vascular pedicle is brought over the cartilaginous support by rotating the fascia over 180°. The rib cartilage framework is thus enclosed by inner and outer vascular fascia layers (Fig 2B). Revascularization occurs by an arterial end-to-end anastomosis with the superior thyroid artery and by a venous end-to-side anastomosis with the internal jugular vein. Flap viability is monitored postoperatively with echo Doppler. Removal of the laryngeal stent and closure of the tracheostomy is done after 1 week.

## MORPHOLOGIC EVALUATION OF COMPOSITE RECONSTRUCTION TECHNIQUE

### RADIOLOGY

An axial gadolinium-enhanced spin-echo magnetic resonance image taken after frontal laryngeal reconstruction reveals a homogeneous enhancement of the inner and outer fascia layers on both sides of the cartilage autograft. A fascia flap fold beyond 180° has no influence on the survival of the inner fascia layer. Although a fascia flap is inherently thin and conformable, the inner fascia with the full-thickness mucosal graft is still much thicker than the mucosal

Fig 5. Histologic evaluation of composite reconstruction technique. A). Reconstruction of anterior laryngotracheal defect in rabbits with composite reconstruction technique (vascularized fascia, full-thickness mucosa, autogenous ear cartilage). Arrows — vocal folds. B) Photomicrograph of quadrangular detail of A (H & E, original ×25). Single arrow—anastomosis between laryngeal and buccal mucosa, 1 — full-thickness oral mucosal graft, 2 — inner fascia layer, 3 — autogenous ear cartilage, 4 — outer fascia layer, 5 — native thyroid cartilage, blue spots — Microfil-injected blood vessels after silicone dye injection of artery of fascia flap.



layer of the normal larynx (Fig 3A). Mucosal grafts may easily be adapted to the remaining laryngotracheal mucosa. The junction between both epithelial linings remains identifiable on magnetic resonance imaging (Fig 3B).

### ENDOSCOPY

The oral mucosa can be distinguished, by its pale appearance, from the surrounding laryngotracheal mucosa (Fig 4). The buccal mucosal translucency allows an inspection of the fascial blood vessels. The transition between buccal and laryngeal mucosae is sharp, without granulations, indicating the full survival of the mucosal graft.

### HISTOLOGY

Histology of the composite reconstruction technique can be obtained from experimental reconstruction. Anterior laryngotracheal defects may be reconstructed in rabbits by means of vascularized fascia, buccal mucosa, and ear cartilage (Fig 5). Histologic comparison of the native airway wall and the reconstructed part reveals

1. A well-healed airway wall anastomosis.
2. A sharp demarcation between oral and respiratory epithelia.
3. A discrepancy in thickness of internal lining between normal and reconstructed larynx and trachea. (Although ultrathin tissues were used, it seems impossible to approach the natural mucosal thickness.)
4. An excellent vascularization of the inner and outer fascia layers on both sides of the cartilage autograft.

### DISCUSSION

Until safe, chronic immune modulation allows for routine allograft transplant replacement of complex, difficult-to-reconstruct composite tissues such as the larynx and trachea, innovations will be needed to refine the reconstruction of these defects. From this perspective, the composite reconstruction technique using a vascular carrier is characterized by the transfer of full-thickness mucosa, cartilaginous support, and abundant blood supply to a laryngotracheal defect in a one-stage procedure. In dealing with laryngotracheal defects, the search for well-vascularized reconstructive tissues is a first requirement. Free grafts of rib cartilage and nasal septum have to rely on the blood supply around the defect for their survival. Revascularization of mucochondral grafts can only be expected for small defects not exceeding 1.5 cm in diameter.[13]

Periosteum and bone may also be used with their own blood supply provided by a muscle[5] or vessel pedicle.[7] They provide a permanent supportive framework without an epithelial lining on which the mucosa can regenerate. Reports of the ultimate fate of these raw surfaces are lacking. Granulation tissue will be formed due to the capillary outgrowth from the uncovered vascularized surface. The frequency of the need for laryngoscopic removal of granulation tissue after reconstruction with periosteum or bone attests to the significance of the problem. This granulation tissue inevitably leads to wound contraction. The contracted wound area will ultimately become resurfaced by respiratory epithelium from the adjacent airway, but the amount of respiratory epithelium migration over raw surfaces is unknown. DeLorimier et al[14] noted that when a graft amounts to 30% or more of the circumference of the airway, exuberant granulation tissue forms and resurfacing from adjacent epithelium does not occur.

Wound contraction may be tolerated inside the larynx and trachea only when tissue loss is small. A cordectomy wound will heal satisfactorily by contraction and epithelialization.[2] Granulation tissue formation and wound contraction are, however, mostly to be avoided in laryngotracheal wounds because of their negative influence on the airway lumen. One of the most effective ways to control the contraction of a laryngotracheal wound is through the application of a full-thickness skin or mucosal graft. Experimental studies on full-thickness skin wounds have shown that the amount of wound contraction, when uncovered, leads to a loss in wound surface area of 80%. Wounds covered by split-thickness skin preserved 30% of their original surface area, while full-thickness grafted wounds preserved their initial shape.[15] Similar data were obtained for laryngeal defects. Full-thickness mucosal grafts reduce the amount of wound contraction by 60%.[11] Full-thickness skin grafts have the same effect on healing as mucosal grafts, but the latter are preferable for laryngotracheal lining because of the absence of hair growth and the reduced tendency for crusting and desquamation. Respiratory mucosal grafts are theoretically superior for airway lining, but are available only in small amounts and are technically intractable as grafting material.

The best way, in our opinion, to bring viable buccal mucosa inside a full-thickness defect is by using a transferable vascular bed. The buccal mucosa will become completely revascularized when sutured carefully to tissues containing a perfused capillary network. Tissues that are potentially useful as a vascular carrier need an axial perfusion provided by an easily transferable vascular pedicle. For laryngotracheal repair it is mandatory to use a carrier with as little bulk as possible. Wide surfaces of thin, pliable fascia that conform to any complex shape and provide a high density of capillaries are useful in providing neovascularity as a recipient graft bed for the mucosal lining. Possible donor sites for fascia flaps are the radial forearm,[16] lateral arm,[17] temporoparietal area,[18] scapular area,[19] and lateral thoracic (serratus) area.[12] We elected the serratus fascia for its large surface area and the consistently long and reliable vascular pedicle, although its dissection is bloody and tedious.

The third component of the composite reconstruction, the autogenous cartilage, provides the necessary external support. The grafts are placed between internal and external fascial sheets — an optimal environment for preserving cartilage viability.

This composite reconstruction using a vascular carrier may be used in selected cases of extended vertical partial laryngectomy defects after resection of anterior commissure tumors with subglottic extension. We prefer the composite reconstruction technique in cases in which the epiglottic pull-down is not sufficient to cover the full extent of the surgical defect or when the epiglottic petiole has to be resected to obtain safe tumor margins. Similar defects were reconstructed satisfactorily with a combination of epiglottoplasty and the sternohyoid myocutaneous rotary door flap.[8] Two disadvantages of this myocutaneous flap prevented its use in our three initial cases undergoing composite reconstruction after salvage partial laryngectomy. The hair-bearing skin island in male patients is not the ideal airway lining, and the vascularity of the sternohyoid flap may be compromised after a radiotherapeutic dosis of 66 Gy, so that its viability after rotation would be uncertain.

Composite reconstruction may also be used in the treatment of tracheal stenosis. Segmental resection with end-to-end anastomosis may be applied successfully in most cases of tracheal stenosis. In a small number of tracheal resections, excessive anastomotic tension may lead to anastomotic stricture, and a re-intervention will be necessary if the stricture is resistant to conservative measures. A re-resection of the stricture may then be considered.[20] A valuable alternative is a longitudinal tracheal incision with wedge augmentation using a viable full-thickness mucosal lining that will augment the lumen and prevent further scar contracture.

Already well-identified reconstruction techniques are appropriate for most laryngotracheal reconstructive problems. Although indications for this composite tissue reconstruction technique will be infrequent, it may be used when the introduction of a well-vascularized mucosal layer seems essential and when simpler approaches are not suitable.

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## FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria (in English July 3-5, 1997, and in German September 4-6, 1997). For more information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax 43/316/385/3425.

*Ann Otol Rhinol Laryngol* 106:1997

# MIGRAINE-RELATED VESTIBULOPATHY

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Migraine has been associated with specific vestibular disorders, including benign paroxysmal vertigo of childhood and benign recurrent vertigo in adults. Migraine may also play a role in chronic nonspecific vestibulopathy. Because scant data exist that describe the clinical findings and vestibular function abnormalities in suspected migraine-related vestibulopathy, we reviewed the history, physical examination, vestibular tests (electronystagmography, rotational chair, posturography), and response to treatment of 100 patients with diagnoses of migraine-related vestibulopathy. Dominant clinical features included chronic movement-associated dysequilibrium, unsteadiness, space and motion discomfort, and occasionally, episodic vertigo as an aura prior to headache, or true vertigo without headache. Common vestibular test abnormalities included a directional preponderance on rotational testing, unilateral reduced caloric responsiveness, and vestibular system dysfunction patterns on posturography. Treatment was usually directed at the underlying migraine condition by identifying and avoiding dietary triggers and prescribing prophylactic anti-migraine medications. Symptomatic relief was also provided using anti–motion sickness medications, vestibular rehabilitation, and pharmacotherapy directed at any associated anxiety or panic disorder.

KEY WORDS — migraine, vestibular disorder.

## INTRODUCTION

Migraine has long been associated with specific vestibular disorders, including benign paroxysmal vertigo of childhood[1-3] and benign recurrent vertigo in adults.[4,5] Basilar artery migraine[6,7] represents a special form of migraine associated with severe vertigo, but chronic, nonspecific symptoms of vestibular system dysfunction can also be related to migraine.[8-10] The manifestations of migraine-related vestibular symptoms are quite varied, ranging from episodic true vertigo to constant imbalance, movement-associated dysequilibrium, and space and motion discomfort. The onset of vestibular symptoms may occur in childhood or adulthood. Symptoms can occur prior to the onset of headache, during a headache, or during a headache-free interval.

Because of the highly varied presentation of symptoms, the diagnosis of migraine-related vestibulopathy requires an awareness of the potential vestibular manifestations of migraine and a meticulous history-taking with specific inquiry into the occurrence of headache and other migraine-associated symptoms and into the family history of migraine. The objective of this paper is to assist clinicians in the diagnosis and management of migraine-related vestibulopathy. With this goal in mind, we review the signs, symptoms, and vestibular test findings of 100 patients with diagnoses of migraine-related vestibulopathy. Our diagnostic guidelines for migraine-related vestibulopathy are outlined, along with the most common profiles of vestibular laboratory test abnormalities. Treatment strategies are discussed.

## METHODS

The subject population for this study consisted of 100 patients identified by retrospective chart review as having a diagnosis of migraine-related vestibulopathy. These patients were identified by a computer search of the medical records of patients presenting with a chief complaint of vestibular dysfunction to the Department of Otolaryngology for the 3-year period between July 1990 and June 1993. A total of 4,400 patients were identified with a vestibular-related diagnosis. A final diagnosis of migraine-related vestibulopathy was found in 100 patients, who form the basis of this study. Each patient had a complete otoneurologic examination, a detailed headache history, vestibular laboratory testing, and brain imaging.

The diagnosis of migraine-related vestibulopathy was based on the following diagnostic features: 1) a history of migraine headaches, 2) a family history of migraine, 3) a history of space and motion discomfort, which includes generalized intolerance to excessive self-motion, motion sickness sensitivity, and susceptibility to visually evoked vertigo, and 4) vestibular symptoms that did not fit other common vestibular syndromes such as Meniere's disease, benign paroxysmal positional vertigo, or vestibular neuronitis.

Headache was considered to be migrainous if it

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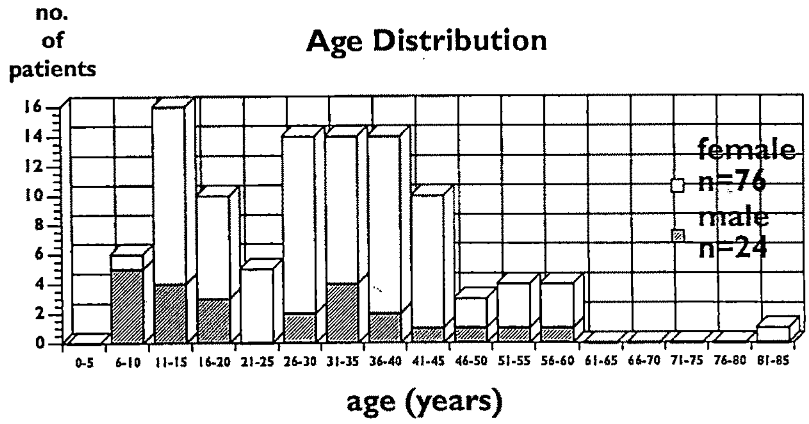

Age distribution of patients with migraine-related vestibulopathy.

was described as pulsatile or throbbing, lasting for several hours, hemicranial, and usually localized, associated with nausea and/or vomiting and photophobia and/or phonophobia. The classification of migraine included migraine with aura (classic migraine), migraine without aura (common), recurrent aura without headache (migraine equivalent), and basilar artery migraine. Migraine with aura was defined as the occurrence of transient neurologic symptom(s) such as visual disturbances (scotoma, blurring, diplopia), paresthesias, motor weakness, or vertigo (without the associated characteristics of basilar artery migraine) followed within 30 minutes by a migrainous-type headache. Migraine without aura was defined as a migrainous-type headache without the preceding aura. Recurrent aura without headache was defined as transient neurologic symptoms (including vertigo) occurring usually but not solely in a child or adolescent with a positive family history of migraine headaches. Basilar artery migraine was defined according to Bickerstaff's criteria,[6] which emphasize the occurrence of transient neurologic deficits related to brain stem or posterior fossa dysfunction. These include a total loss of vision or visual manifestations throughout both visual fields, vertigo, ataxia of gait, dysarthria, or motor weakness, often of a hemiparetic type. The headache that followed was described as usually occipital rather than hemicranial, with or without impairment of consciousness, stupor, restlessness, or complete loss of consciousness. We did not consider patients with vertigo during the aura phase of migraine occurring without other symptoms of brain stem dysfunction to have basilar artery migraine.

Vestibular laboratory testing consisted of an ocular motor screening battery including a search for nystagmus both with and without fixation, and with horizontal and vertical gaze deviation, and an assessment of saccades, smooth pursuit, and optokinetic nystagmus. Static position nystagmus was studied in the supine, head right, head left, right lateral, and left lateral positions with the eyes open in the dark. Caloric function was studied with use of alternate binaural bithermal caloric irrigation with a closed-loop irrigator with water at 30°C and 44°C. In our laboratory, a normal response is considered to be a vestibular response asymmetry of less than 25% and a directional preponderance of less than 30%.

Earth-vertical axis rotational chair testing was performed with eyes opened behind darkened goggles with the use of electrooculography. Eye movement signals were amplified by a DC-coupled amplifier with a cutoff frequency of 40 Hz. The amplified and filtered eye movements were recorded on chart paper. Sinusoidal stimulation used single-frequency sinusoids of 0.05, 0.1, 0.5, and 1.0 Hz with a peak amplitude of 50°/s. In addition, an amplitude series at 0.1 Hz was performed with peak amplitudes of 25°/s, 50°/s, 100°/s, and 150°/s. The DC bias and gain symmetry of the resulting eye movements was calculated and used to determine directional preponderance. Some patients also underwent velocity trapezoids that consisted of a slow angular acceleration of $1.5°/s^2$ to a constant velocity of 60°/s, which was maintained for 60 seconds. The chair was then rapidly decelerated at $100°/s^2$ to stop the rotation. The peak amplitude and long time constant of the resultant nystagmus were calculated.

The vestibulospinal system of the patients was evaluated by computerized moving platform posturography (EquiTest; Neurocom, Clackamas, Ore).

## RESULTS

The population of 100 studied included 76 women and 24 men. The age of onset of vestibular symptoms ranged from 7 to 83 years old. The age distribution of patients is summarized in the Figure. Fifty patients presented for evaluation within the first year after onset of dizziness. Thirty patients had had their symptoms more than 1 year but less than 5 years, while 20 had had their symptoms more than 5 years before

TABLE 1. SUMMARY OF PREDOMINANT TRANSIENT NEUROLOGIC SYMPTOMS (AURA) ASSOCIATED WITH CLASSIC MIGRAINE (N = 50)

| | *No. of Patients* |
|---|---|
| Scintillating scotoma | 22 |
| Blurred vision | 11 |
| Visual field cuts | 3 |
| Double vision | 3 |
| Paresthesias | 5 |
| Vertigo | 6 |

presenting for evaluation.

The type of migraine headache was classified as migraine with aura (classic) in 50 patients, migraine without aura (common) in 46 patients, and aura without headache (migraine equivalent) in 4 patients. No patient fulfilled the criteria for basilar artery migraine.

A summary of the predominant transient neurologic symptoms (aura) associated with classic migraine in 50 patients is summarized in Table 1. Six patients reported vertigo with a duration and temporal relationship to headache typical of a migrainous aura. Since these patients had only a single focal neurologic symptom, we felt it was more logical to regard these patients as having migraine with vertigo as an aura instead of saying they had basilar artery migraine, since their symptoms did not fulfill the diagnostic guidelines outlined by Bickerstaff.[6]

A family history of migraine was present in 77 patients, while 18 denied a family history of migraine. The family history was not available for 5 patients.

Space and motion discomfort was reported by 77 patients. Sixteen patients reported no space and motion discomfort, and in 7 patients the severity of this symptom was unknown.

The character of vestibular symptoms is summarized in Table 2. The predominant symptom complex reported by 79 patients was movement-associated dysequilibrium, lightheadedness, and unsteadiness. Many of these patients reported an illusion of movement (vertigo) occurring only during active movements. The duration of these symptoms was quite varied, with nearly equal numbers of patients having symptoms lasting hours, symptoms lasting days to weeks, or constant symptoms. Episodes of spontaneous true vertigo were reported by 21 patients. The true vertigo lasted hours in most of the patients and preceded the headache as an aura in 6 of the patients.

TABLE 3. RESULTS OF ELECTRONYSTAGMOGRAPHY IN MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | *No. of Patients* |
|---|---|
| Normal | 61 |
| Unilateral reduced vestibular response | 18 |
| Bilateral reduced vestibular response | 4 |
| Spontaneous nystagmus | 7 |
| Directional preponderance | 5 |
| Direction-changing positional nystagmus | 7 |
| Direction-fixed positional nystagmus | 6 |
| Impaired pursuit | 3 |
| Impaired optokinetic nystagmus | 2 |

Some patients had more than one abnormality.

TABLE 2. CHARACTER OF SYMPTOMS IN MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | | *No. of Patients* |
|---|---|---|
| Movement-associated dysequilibrium, lightheadedness, and unsteadiness | | 79 |
| Seconds to minutes | 14 | |
| Hours | 41 | |
| Days to weeks | 25 | |
| Always | 34 | |
| True vertigo | | 21 |
| Seconds | 10 | |
| Minutes | 38 | |
| Hours | 52 | |

The results of electronystagmography are summarized in Table 3. It should be noted that the majority of patients (61) had a normal electronystagmography result. Only 18 patients demonstrated a unilateral reduced vestibular response, and 4 demonstrated a bilaterally reduced vestibular response. Central nervous system dysfunction on the ocular motor screening battery including defective smooth pursuit and optokinetic nystagmus was rarely observed. Table 4 summarizes the results of rotational chair testing. Nearly half the patients (49) showed an abnormal directional preponderance on rotational chair testing. The results of posturography are summarized in Table 5. Most patients (74) had normal posturography. In the patients with abnormal posturography there was a mixture of patterns, but most included the vestibular patterns involving conditions V and VI.

The most common patterns of abnormalities on vestibular tests, including electronystagmography,

TABLE 4. RESULTS OF ROTATIONAL CHAIR TESTING IN MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | *No. of Patients* |
|---|---|
| Normal | 48 |
| Directional preponderance | 49 |
| Interrupted | 2 |
| Reduced gain | 1 |

TABLE 5. RESULTS OF POSTUROGRAPHY IN MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | *No. of Patients* |
|---|---|
| Normal | 74 |
| Abnormal patterns | |
| Nerve V | 2 |
| Nerve VI | 6 |
| Nerves V, VI | 7 |
| Nerves IV, V, VI | 3 |
| Nonspecific | 8 |

Abnormal posturographic results were only abnormality on vestibular testing in 4% of patients.

rotational chair testing, and posturography, are summarized in Table 6. Only 27 patients had completely normal results on vestibular function testing. A directional preponderance on rotational chair testing, alone or in combination with a reduced vestibular response on caloric testing, or an abnormal posturography result was seen in a total of 49 patients. A directional preponderance on rotation alone or in combination with other abnormality thus stands out as the most characteristic vestibular test abnormality in patients with migraine-associated vestibulopathy. No significant relationship was found between the character of reported symptoms and vestibular test results.

Audiometry was normal in 80 patients, 3 patients demonstrated a mild low-frequency sensorineural hearing loss, 5 patients demonstrated a high-frequency sensorineural hearing loss, 1 patient demonstrated a conductive hearing loss, and 11 patients were not tested. Fifty-nine patients reported no associated aural symptoms. Aural fullness was noted by 28 patients. This was unilateral in 11 patients and bilateral in 17 patients. Tinnitus was reported by 26 patients. The tinnitus was unilateral in 7 patients and bilateral in 19 patients.

Imaging studies consisting of magnetic resonance imaging and/or computed tomography were performed in 76 patients. The results of these studies were all normal, except in 1 patient who had an incidental 1-mm Chiari malformation, 1 patient with a posterior fossa subarachnoid cyst, and 3 patients with sinusitis. None of these findings were thought to contribute to the patients' symptoms. The reported secondary diagnoses are summarized in Table 7.

## DISCUSSION

Migraine is a common disorder, with an estimated prevalence in the United States of 17% to 29% of adult women and 6% to 20% of adult men.[11,12] Dizziness is a common feature of migraine and is estimated to occur in 25% of unselected migraine patients,[8] and the incidence of vestibular symptoms in patients with migraine headaches has been shown to be significantly greater than that in patients with tension headache[8] or headache-free subjects.[9] The general term for dizziness associated with migraine — migraine-related or -associated dizziness[10] — encompasses the overall relationship between migraine and vertigo, as well as a number of previously described vestibular syndromes, including basilar artery migraine, benign paroxysmal vertigo of childhood, and benign recurrent vertigo.

TABLE 6. MOST COMMON PATTERNS OF VESTIBULAR TEST ABNORMALITIES IN MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | *No. of Patients* |
|---|---|
| Normal | 27 |
| Isolated directional preponderance on rotation | 22 |
| Directional preponderance on rotation and reduced vestibular response | 12 |
| Directional preponderance on rotation and abnormal posturography results | 9 |
| Directional preponderance on rotation, reduced vestibular response, and abnormal posturography results | 6 |
| Isolated reduced vestibular response | 5 |
| Isolated abnormal posturography results | 4 |

Including electronystagmography, rotational chair testing, and posturography.

*Basilar Artery Migraine*. Bickerstaff[6] first associated vertiginous symptoms preceding migraine with dysfunction of the brain stem and other areas within the distribution of the basilar artery and coined the term basilar artery migraine. In Bickerstaff's original description of 34 patients, all but 2 were under the age of 23, and 26 were adolescent girls. The initial symptom was usually visual, consisting of either a total loss of vision or visual manifestations throughout both visual fields. This was followed by vertigo, ataxia of gait, dysarthria, and motor weakness, often of a hemiparetic type. The headache that followed

TABLE 7. SECONDARY DIAGNOSES REPORTED BY PATIENTS WITH MIGRAINE-RELATED VESTIBULOPATHY (N = 100)

| | *No. of Patients* |
|---|---|
| Anxiety or panic disorder | 14 |
| Sinusitis | 5 |
| Seizure disorder | 4 |
| Head injury | 4 |
| Depression | 2 |
| Connective tissue disorder | 2 |
| Trigeminal neuralgia | 1 |
| Perilymphatic fistula | 1 |
| Subarachnoid cyst | 1 |

was described as usually occipital rather than hemicranial. In addition, impairment of consciousness with stupor or restlessness or complete loss of consciousness occurred in a number of the patients.

Review of the literature shows variability in the use of the term basilar artery migraine. Olsson[13] and Harker and Rassekh[14] classified cases of a history of headache and symptoms of auditory and vestibular dysfunction as basilar artery migraine. However, their published case examples bear only partial resemblance to Bickerstaff's[6] or Eviatar's[7] classic descriptions of basilar artery migraine. Of interest, 80% of patients reported by Olsson had a low-frequency hearing loss, which is also characteristic of Meniere's disease. While an association between Meniere's disease and migraine is possible, we prefer to consider true basilar artery migraine as a separate entity.

None of the patients reviewed in this series presented as classically described by Bickerstaff.[6] The patients closest to Bickerstaff's description were those having the occurrence of vertigo during the aura phase of migraine. However, none of these patients displayed any of the other associated symptoms described by Bickerstaff. Thus, we chose to categorize these patients, as did Cutrer and Baloh,[10] as having migraine with aura (vertigo).

*Benign Paroxysmal Vertigo of Childhood.* A second clinical syndrome of dizziness associated with migraine is benign paroxysmal vertigo of childhood, described by Basser[1] in 1964. Basser described a particular variety of paroxysmal vertigo occurring in childhood, with the cardinal symptom of vertigo occurring in isolation. There is a characteristic absence of aural symptoms, tinnitus, or hearing loss. The age of onset is usually within the first 3 or 4 years of life, but may be later, for example, at age 7 or 8. The spells of vertigo are reported to be brief, lasting seconds, usually less than a minute and rarely more than a few minutes. Pallor, nausea, sweating, and occasional vomiting occur. Consciousness is not impaired during the attacks, and there is no pain or headache associated with the attacks. Frequently, these children demonstrate a unilateral reduced vestibular response to caloric testing. While Basser did not associate this syndrome with migraine, others, including Koehler[2] and Lanzi et al,[3] were able to follow children with benign paroxysmal vertigo in a longitudinal fashion and confirm an association with migraine. Children with benign paroxysmal vertigo of childhood often have a positive family history of migraine, often subsequently develop migraine headache in later years, and can respond positively to antimigrainous treatment.

The present series of patients included 42 children and adolescents between the ages of 6 and 20 years. However, unlike those in Basser's[1] series, most of our patients did not report true vertigo, but more commonly described paroxysmal lightheadedness, imbalance, and movement-associated dysequilibrium; and only 8 of the patients demonstrated a reduced vestibular response on caloric testing. These differences suggest that our population may have been less severely affected than Basser's patients or that our patients had a somewhat different form of migraine-related vestibulopathy. Nevertheless, we use the diagnosis of benign paroxysmal vertigo of childhood to describe these children and adolescents.

*Benign Recurrent Vertigo.* A third vestibular syndrome associated with migraine is the entity of benign recurrent vertigo described by Slater[4] in 1979. Slater used the term benign recurrent vertigo to describe patients with acute attacks of spontaneous vertigo not precipitated by movement and not accompanied by other neurologic or auditory symptoms, occurring in young or middle-aged adults. The attacks of vertigo can last hours to days and are followed by variable periods of dysequilibrium. No aural symptoms or hearing loss accompany the attacks, and results on caloric testing are normal. Slater stressed that patients with this syndrome showed many features in common with migraine, including precipitation by alcohol, lack of sleep, and emotional stress; a female preponderance; and positive family histories of migraine.

Slater's[4] description is most consistent with the patients we reviewed that had symptoms during the headache-free period (44 patients) or without headache at all (4 patients). Most of these patients had normal caloric function. However, only 7 of these patients reported true episodic vertigo, and the majority described more nonspecific symptoms such as paroxysmal lightheadedness, imbalance, and movement-associated dysequilibrium. Thus, overall, only 7 cases closely resembled Slater's original description of benign recurrent vertigo.

*Migraine-Related Vestibulopathy.* Many of the cases we reviewed did not clearly fall into the well-defined migraine-related clinical syndromes described above, but shared many common characteristics that define migraine-related vestibulopathy in general. These characteristics include highly variable vestibular symptoms, a positive history of migraine headaches, a positive family history of migraine, a history of space and motion discomfort, abnormal findings on vestibular function testing (most commonly a directional preponderance on rotational testing), and the absence of hearing loss and auditory symptoms. In addition, none of these patients had diagnoses of well-defined vestibular syndromes such as Meniere's disease, benign paroxysmal positional vertigo, or vestibular neuronitis.

In our series of patients with migraine-related vestibulopathy, 79 reported symptoms of movement-associated dysequilibrium, lightheadedness, and imbalance. Only 21 patients described symptoms that could be classified primarily as vertigo. The prevalence of true episodic vertigo that we report is less than that reported by other investigators. For example, Kayan and Hood[8] divided their patients' vestibular symptoms into "vertigo," defined as an illusion of rotation of the environment or of the patient himself or herself, and "giddy sensations," defined as less well-defined vestibular disturbances described as dizziness, giddiness, a swimming sensation in the head, a floating or sinking sensation, or transitory feelings of being off balance. Kayan and Hood reported that approximately half of patients reported vertigo, with the other half reporting only giddy sensations. Even if the 15% of patients with Meniere's disease and benign paroxysmal positional vertigo that are included in the Kayan and Hood series are excluded, there remain approximately 35% of their patients with episodes of true vertigo. Cutrer and Baloh[10] also divided reported symptoms into vertigo and nonvertiginous dizziness such as severe imbalance, nausea, and sensitivity to motion. They found that 32% of subjects reported both types of symptoms. Thirty-one percent experienced nonvertiginous dizziness, and 37% of patients reported solely symptoms of episodic vertigo. Despite these apparent differences in the reported symptoms of true vertigo, the prevalence of a unilateral reduced vestibular response (RVR) is markedly similar among previous studies and the present one. Kayan and Hood reported that 24% of patients had an RVR, Cutrer and Baloh reported 21% with an RVR, and 18 patients (18%) in this review had an RVR.

Another characteristic feature of migraine-related vestibulopathy is a history of space and motion discomfort.[15] This condition manifests as a generalized intolerance to excessive self-motion (motion sickness) and susceptibility to visually evoked vertigo and was reported by 77 patients in this review. The tendency for motion sickness has been shown to occur significantly more frequently in patients with migraine headaches as compared with nonheadache controls (87% versus 11%).[9]

In our series, the most common vestibular test abnormality in migraine-related vestibulopathy is a directional preponderance on rotational chair testing (seen in 49 patients). This characteristic finding was first noted by Toglia et al[16] and also observed by Cutrer and Baloh.[10] Of interest, despite the frequent complaint of imbalance and unsteadiness, findings on posturography were usually normal.

Audiometry was performed in 80% of the patients in our study. Two patients had a low-frequency hearing loss consistent with endolymphatic hydrops. However, these patients did not have the associated symptoms of episodic aural fullness, tinnitus, fluctuating hearing loss, and true vertigo that define Meniere's syndrome. Moreover, electrocochleographic findings were normal in these 2 patients. Although a relationship between migraine and Meniere's disease has been proposed,[13] it is our experience that the relationship seems to be more of a chance rather than a positive association.[10,17] Thus, in our diagnostic criteria for migraine-related vestibulopathy, patients with clear-cut Meniere's syndrome are excluded unless the episodes are definitively time-locked to migraine headache.

An anxiety or panic disorder was the most common secondary diagnosis, reported by 14 patients. Four patients were also under treatment for a seizure disorder that we felt was not related to the migrainous disorder or vestibular symptoms. Four patients also reported mild head injury in the past. All 4 patients had a previous history of migraine headaches, and although these patients reported having more symptoms following their head injury, we did not feel that these patients' symptoms were primarily due to the head injury. Two patients carried a diagnosis of a connective tissue disorder. A diagnosis of an autoimmune inner ear disorder usually rests upon the presence of audiologic and vestibular symptoms with a positive response to steroid therapy. The 2 patients included here did not have auditory symptoms, the vestibular symptoms did not respond to steroids, and the vestibular symptoms were clearly related temporally to migraine headaches. In the 1 patient with trigeminal neuralgia, we raised the question whether or not the vestibular symptoms could be due to vascular cross-compression of the eighth nerve. However, because of the temporal association of vestibular symptoms with headache and a positive response to antimigrainous therapy, we are comfortable with a primary diagnosis of migraine-related vestibulopathy in this patient. One patient had surgery for exploration of a suspected perilymphatic fistula. However, the exploration was negative, and thus the diagnosis of perilymphatic fistula could not be confirmed.

The frequent association of anxiety and panic disorders in our patients highlights the similarities in symptoms between psychogenic dizziness and migraine-related vestibulopathy. The coexistence of both conditions in many patients and similarities of

TABLE 8. TREATMENT STRATEGIES FOR MIGRAINE-RELATED VESTIBULOPATHY

- Avoid dietary triggers
- Treat underlying migraine phenomenon
  - Tricyclic antidepressants (eg, amitriptyline hydrochloride 50 to 100 mg/d)
  - Beta-blockers (eg, propranolol 80 to 320 mg/d)
  - Calcium channel blockers (eg, verapamil hydrochloride 80 to 120 mg/d)
- Treat movement-associated dysequilibrium
  - Vestibular rehabilitation therapy
- Treat space and motion discomfort
  - Phenergan/pseudoephedrine (25 mg/60 mg twice daily)
  - Valium
- Treat associated anxiety or panic disorder
  - Behavioral therapy
  - Pharmacotherapy
    - Tricyclic antidepressants
    - Anxiolytics, eg, benzodiazepine

the symptoms, including the common occurrence of space and motion discomfort, raises the question whether both conditions share a related underlying mechanism of vestibular system dysfunction.

Clinical observations of migraine-related vestibulopathy have reported that vestibular symptoms occur both as short-duration attacks of vertigo that precede a headache and as a longer-duration condition of imbalance, movement-associated dysequilibrium, and motion sickness that can persist for days or weeks after the headache or during a headache-free period.[8-10] Cutrer and Baloh[10] hypothesized that the short-duration vertiginous episodes are a sensory "aura" phenomenon, produced by the same central mechanisms as other auras, and that the longer-duration vestibular symptoms are produced via peptidergic mechanisms. However, they proposed only one specific potential mechanism: a peripheral action with, possibly, a concurrent central action of calcitonin gene–related peptide.

There is a developing consensus in the literature that the actions of serotonin (5-HT) play an important role in the genesis of migraine, through either a direct action on cerebral vasculature, effects on neuropeptide release, or direct actions in the central nervous system.[18-20] In particular, 5-HT–induced vasodilation of cerebral vasculature is believed to be associated with the headache phase of migraine attacks.[20] A role of serotonergic transmission in the headache phase is of interest because 5-HT has direct effects on the firing rate of vestibular nucleus neurons.[21,22] Further, immunohistochemical studies have revealed a prominent serotonergic input to the vestibular nuclei, concentrated heavily in the caudal aspect of the medial vestibular nucleus.[23] Since direct projections to the nucleus of the solitary tract and dorsal motor vagal nucleus arise from the caudal medial vestibular nucleus,[24] changes in 5-HT (eg, asymmetric or activation) have the potential to affect both somatic and autonomic activity. Thus, serotonergic transmission is a possible substrate for explaining the appearance of vertigo during the headache phase of migraine. The longer-duration vestibular symptoms, though, may reflect an interaction of both serotonergic and peptidergic mechanisms. In addition to direct effects, 5-HT can affect the release of neuropeptides, since central injections of a variety of neuropeptides (eg, endothelin-1, arginine vasopressin, somatostatin, oxytocin, met-enkephalin, and dynorphin) elicit acute vestibular dysfunction in experimental animals.[25]

How could these neurochemical substrates cause vertigo? Two possible hypotheses arise. First, asymmetric activation of serotonergic and peptidergic pathways could produce vertigo by producing asymmetric activation of vestibular circuits. Second, it is generally presumed that central vestibular circuits are dynamically compensated for developmental asymmetries in both the peripheral and central nervous system. Hence, symmetric activation of serotonergic and peptidergic pathways may produce spontaneous decompensation, so that latent asymmetries become manifest.

## CONCLUSIONS

Despite the high prevalence of migraine in the general population and the common association with dizziness, the diagnosis of migraine-related vestibulopathy is often overlooked. It is important to correctly diagnose migraine-related vestibulopathy because specific treatment strategies can then be utilized. Our treatment approach is outlined in Table 8. First, it is necessary to educate the patient regarding the association of dizziness with his or her underlying migrainous condition and the importance of avoiding dietary triggers such as tyramine-containing foods, alcohol, and caffeine and avoiding stress and fatigue. Second, one should treat the underlying migrainous condition with prophylactic medications even if headaches are not currently prominent. Our experience is that one third to one half of patients will respond favorably to the types of medications[26] listed in Table 8. If the initial medication is unsuccessful, then other classes of agents should be tried in turn. Third, if the most prominent vestibular symptom is movement-associated dysequilibrium, then aggressive vestibular rehabilitation therapy is recommended. We treated 10 patients with vestibular rehabilitation therapy, and 7 of the 10 patients reported significant benefit from the therapy. Fourth, if the patient reports severe space and motion discomfort, then we use anti–motion sickness medications such as the combination of Phenergan and pseudoephedrine or a mild vestibular suppressant agent such as low-dose Valium. Finally, in patients with panic attacks or agoraphobia we obtain psychiatric consultation and rely on both behavioral therapy and specific medical therapy using tricylic antidepressants or anxiolytic medications.

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The Twenty-Fourth Ordinary Congress of the International Neurootological and Equilibriometric Society Reg. (NES) will be held April 6-10, 1997, in Haifa, Israel. For information, contact NES Congress President Prof Dr Ludwig Podoshin, Otolaryngology, Head and Neck Surgery, Bnai Zion Medical Center, PO Box 4940, Haifa 31048, Israel; telephone 972-4-359544; fax 972-4-371393.

# “ENDOLYMPHATIC SACITIS” IN A CASE OF ACTIVE MENIERE’S DISEASE

## AN ULTRASTRUCTURAL HISTOPATHOLOGIC INVESTIGATION

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An ultrastructural analysis of an entire intraosseous endolymphatic sac (ES) from a patient with active, well-documented Meniere’s disease was performed for the first time. The results were compared with those obtained from ES biopsy material from patients with acoustic neuromas. The ES was small in size and showed signs of focal inflammation with intraepithelial invasion by mononuclear cells. At these places the normal fine structure, including the vascular anatomy, was altered. The possible relationship between these changes and Meniere’s disease is discussed.

KEY WORDS — endolymphatic sac, immune pathology, Meniere’s disease, stereocilia, ultrastructure.

## INTRODUCTION

The cause of Meniere’s disease (MD) still remains obscure. Experimental, anatomic, and physiological studies suggest that the endolymphatic duct (ED) and endolymphatic sac (ES) play a role in the development of endolymphatic hydrops and MD.[1-3]

The light microscopic and histopathologic studies performed so far have indicated that perisaccular fibrosis and reduced vascularity may occur in MD, but the results are still contradictory.[2,4-7] This may be due to the fact that complete biopsy specimens of temporal bones are difficult to obtain in active stages of the disease. This is also the reason why complete histopathologic studies of the ES and ED, at the ultrastructural level, have not yet been performed in well-documented and active cases of MD.

In the following report the results of a transmission electron microscopy (TEM) analysis of a complete intraosseous ES, from a patient with well-documented, active MD, are presented for the first time. The results indicate that the ES was pathologically altered in this case.

## MATERIAL AND METHODS

### PATIENT DATA

The patient was a 64-year-old mechanical engineer with no heredity for ear disease but who had been exposed to heavy noise during his working life. He had had no other disease prior to the first spell of rotatory vertigo with nausea, hearing loss, and tinnitus in his left ear, at the age of 49. The spell lasted for 2 hours. Audiograms showed bilateral sensorineural hearing loss at high frequencies that was more pronounced in the left ear. Since the age of 51 he had suffered from the typical Meniere’s triad with varying frequency of vertiginous spells, aural tinnitus, and fluctuating low-tone sensorineural hearing loss. At the age of 56 he experienced three episodes of vertigo in 1 year, each lasting 2 to 4 hours. One year later he had seven spells of disabling vertigo in 1 month.

The tinnitus was graded as II-III, with sleep disturbances. Meniere’s disease was diagnosed at that time. The audiogram showed sensorineural hearing loss in the left ear for both low and high tones (Fig 1). The hearing levels for low tones were restored to near-normal after ingestion of glycerol (positive glycerol test).[8] Findings on petrous computed tomography scans were normal. The caloric response was decreased on the left side, with a side difference varying from 28% to 52%. The speech discrimination score was 60% on the left side and 100% on the right. Medication with diuretics had little effect on the disabling vertigo, to which general unsteadiness and positional vertigo were added with time. At the age of 59, when the patient showed a flat loss audiogram and the hearing level was about 60 dB (Fig 1), a surgical ultrasound procedure, to destroy the vestibular sensory epithelium, was carried out with 3.5 W for 17 minutes on the three semicircular canals. The surgery was repeated 2 years later and the patient was

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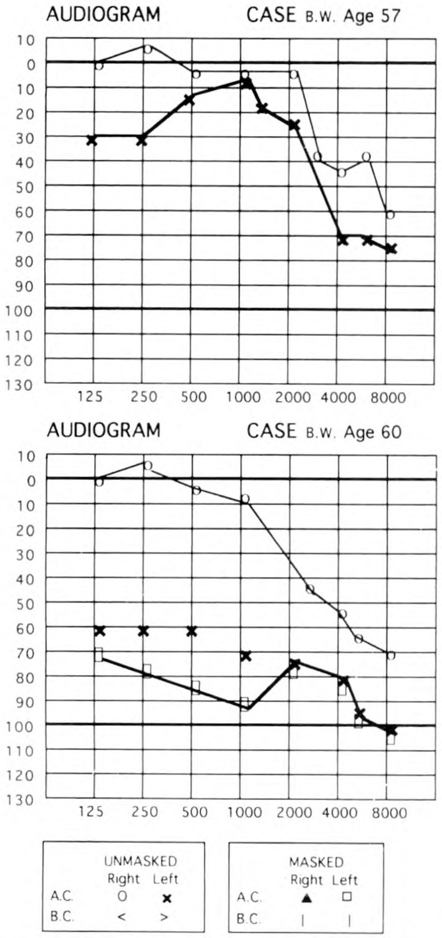


Fig 1. Audiograms taken at ages 57 and 60 years in patient with Meniere's disease. First attack of vertigo occurred at age 49. During 3 years that passed between upper and lower audiograms, hearing in left ear deteriorated to flat loss type of curve.

well until the age of 63 years, when severe attacks of vertigo and vomiting recurred. Two months later a total labyrinthectomy was performed. Postoperatively the patient had a quick recovery with no further spells of vertigo.

### SPECIMEN PROCESSING

During labyrinthectomy the entire labyrinthine block was drilled away and the vestibular aqueduct was identified, skeletonized, and taken out with an intact shell of bone around it (Fig 2). The specimen was then processed for light microscopy and TEM according to previously described methods.[9] Thin sections were viewed in a JEOL electron microscope (JSM 100X).

### CONTROL SPECIMENS

Twenty-two specimens of human intraosseous ES obtained during translabyrinthine surgery for removal of acoustic neuromas (not always affecting the inner ear or eighth nerve function), processed in the same way as above, were used as controls.

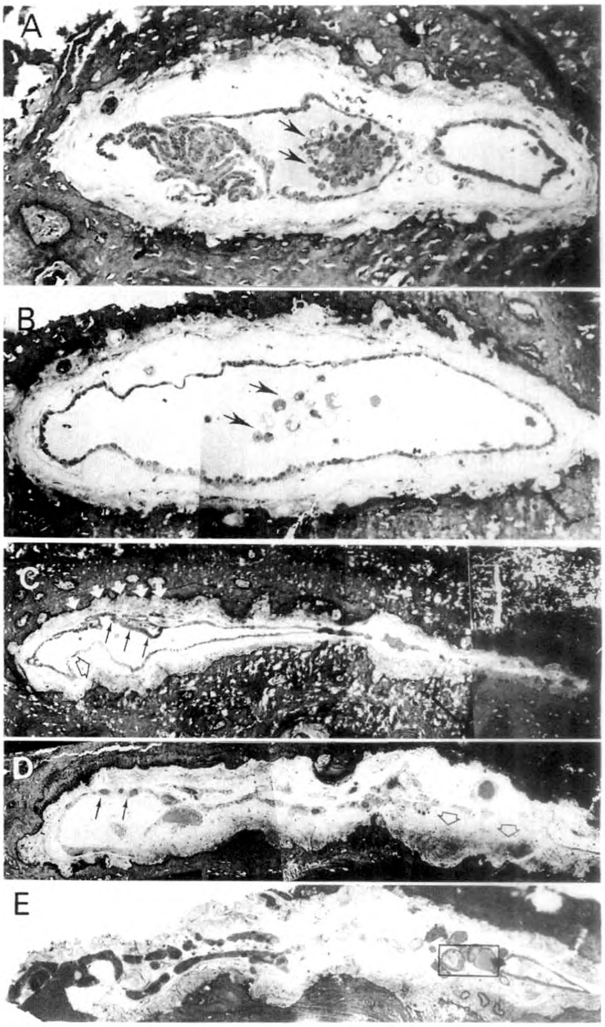


Fig 2. Light micrographs of endolymphatic duct (ED) and intraosseous endolymphatic sac (ES). A) Macrophages and mononuclear cells form aggregations in luminal space of region between distal ED and proximal ES (arrows; original ×220). Size of ES — 0.09 × 0.57 mm. B) Slightly more distal part of ED-ES region (original ×220). Luminal macrophages and monocytes are less numerous (arrows). Size of ES — 0.09 × 0.6 mm. C) Proximal part of ES (original ×120). Few intraluminal cells are discerned. Thin arrows — area of lymphocyte infiltration, open arrow — psammoma body seen subepithelially, size of ES — 0.14 × 1.2 mm. D) Part of intraosseous ES near opercular region (original ×110). Note tubular structures filled with densely staining material. Thin arrows — portion in which some lymphocytes were infiltrating and pathologic changes in basal lamina were seen, open arrows — more dense connective tissue, length of ES — 1.3 mm. E) Part of intraosseous ES at level close to D (original ×110). Framed area — tubules in which many filamentous, circular structures of about 130 to 170 nm in diameter could be seen.

## RESULTS

### LIGHT MICROSCOPY FINDINGS

Both the ED and ES were present in the specimen. The ES was smaller than in most control specimens. The estimated width of the ES close to the operculum was 3 mm. The ES was well preserved, with an intact epithelial wall and with good preservation of the

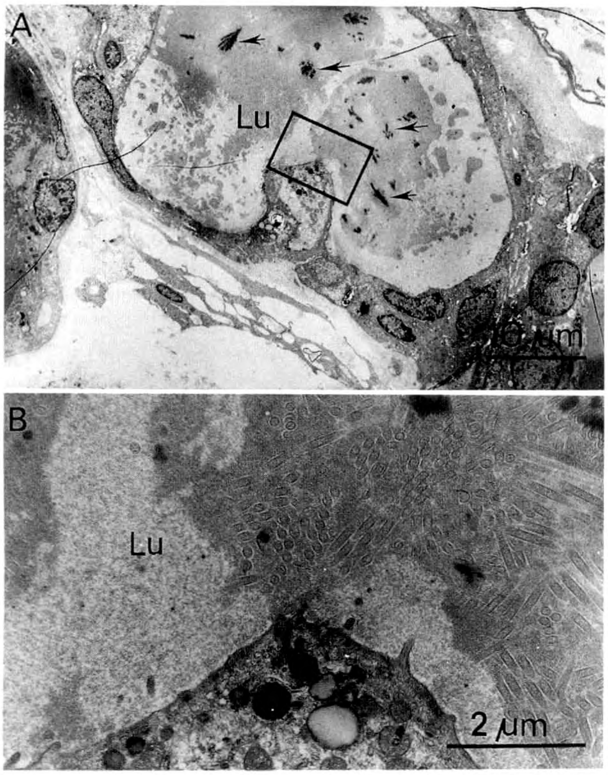


Fig 3. Transmission electron microscopy (TEM) image of ES tubule close to external aperture (lumen [Lu]). In heterogeneously stained luminal contents, scattered or aggregated tubular or spherical structures are seen. Their occurrence was often limited to areas with more densely staining luminal precipitate. A) Tubule of framed area in Fig 2E, showing aggregations of tubules as darkly staining luminal particles (arrows; original ×2,500). B) Same framed area shown at higher magnification revealing spherical or filamentous structures confined to dense luminal precipitate (original ×14,000).

intraluminal contents (Fig 2). The lumen in the transitional zone between the distal ED and proximal ES was filled with a floccular and moderately stained material together with aggregates of macrophages and mononuclear cells (Fig 2A,B).

The epithelium often showed structural polymorphism, as is normally seen in the human ES. The perisaccular tissue seemed to be intact, with a fairly normal number of blood vessels. Psammoma bodies were observed (Fig 2C). In the peripheral regions the connective tissue appeared more dense (Fig 2C-E).

Close to the opercular level the ES consisted of multiple glandularlike acini or tubules of various sizes with a dense intraluminar content (Fig 2D,E).

### TRANSMISSION ELECTRON MICROSCOPY FINDINGS

*Intraluminar Space.* The macrophages in the ED-ES region were found to be active and of the dendritic type, with an abundance of membrane-bound intracytoplasmic vesicles with both a granular and a dense homogeneous content.

In several tubules near the opercular region many filamentous, circular structures, approximately 130 to 170 nm in diameter, sometimes with a length of several micrometers, were observed. These filaments were sometimes arranged in aggregates or more sparsely scattered and were often associated with the more dense intraluminal substance (Figs 2E, 3, and 4). These structures had a dense outer wall about 12 to 15 nm in thickness and a more electron-lucent core (Fig 4). The filaments were surrounded by a thin electron-lucent zone (Fig 4). Such filaments were also found in one of the control specimens.

In the area close to the operculum, the ES consisted of several small tubules with only a few surrounding epithelial cells. In some places, minuscule tubules surrounded by only two to three epithelial cells could be seen.

*Epithelium.* Focally, in the proximal region of the specimen, the ES epithelial layer was invaded by mononuclear lymphoid cells, consisting of small and medium-sized (5 to 10 µm) and large granular and nongranular lymphocytes (10 to 20 µm) and monocytes and macrophages (15 to 30 µm; Fig 5). The latter cells could be identified through their content of multivesicular bodies or rough membranes, sug-

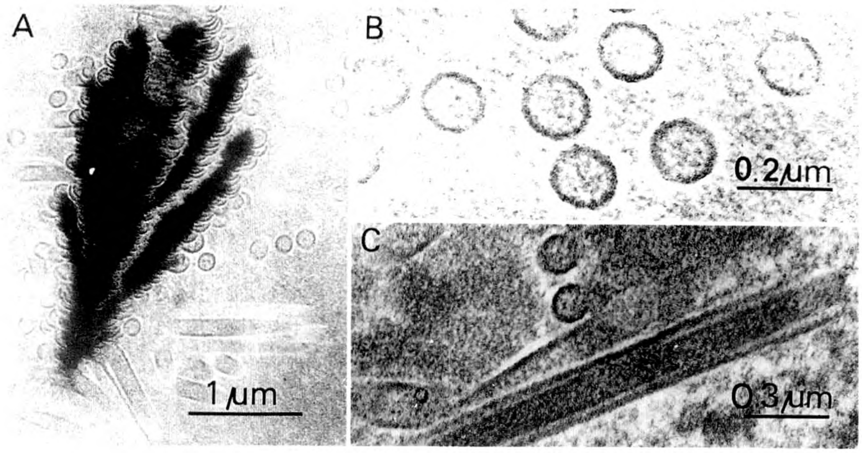


Fig 4. TEM images of luminal structures from Fig 3 at higher magnification. A) Particles are often aggregating closely into ramifying bundles (original ×31,000). B,C) Particles at higher magnification show 12- to 15-nm limiting membrane and granular contents varying in electron density. B) Original ×128,000. C) Original ×75,500.

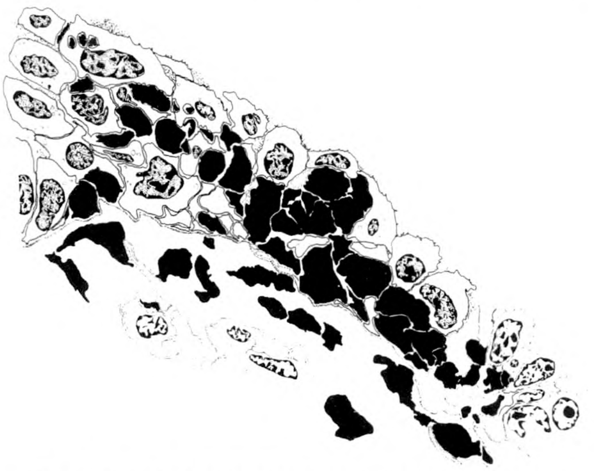


Fig 5. Graphical representation of electron micrograph reconstruction of intraosseous ES from Meniere's patient (original ×1,500). Note abnormal intraepithelial invasion by lymphoid cells. Black cells —mononuclear lymphoid cells, dotted line — basal lamina.

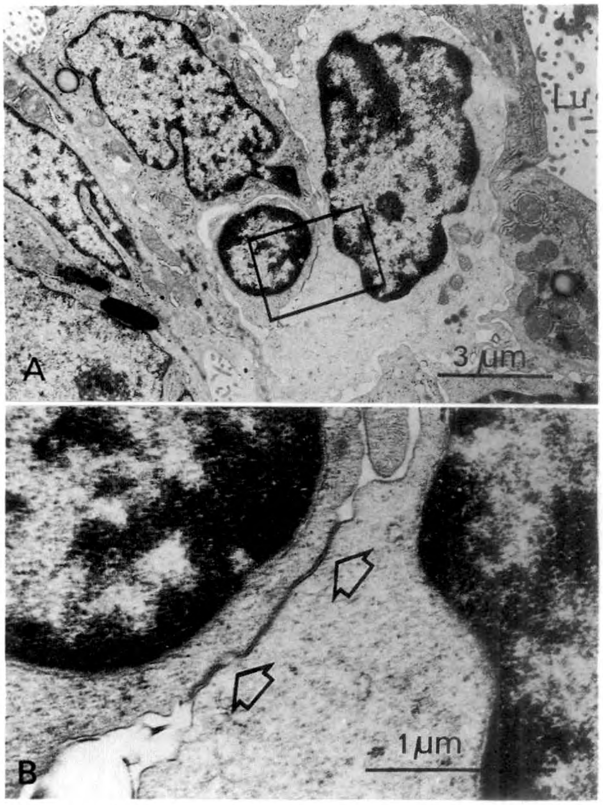
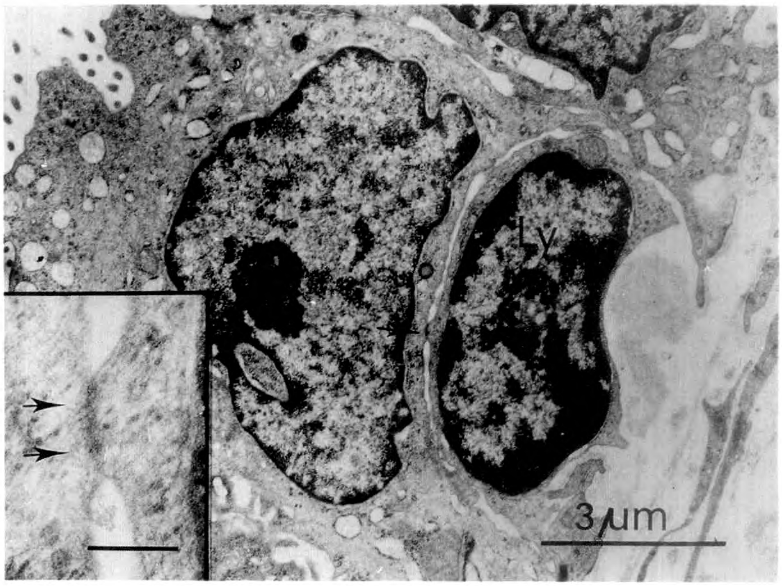


Fig 6. TEM images of physical interaction between two intraepithelially located lymphoid cells. A) Framed area shows cells (original ×9,500). Lu — lumen of ES. B) Framed area of A at higher magnification (original ×50,400). Between two cells there is nonspecific contact site measuring <20 nm (arrows).

gesting a phagocytic nature or a lysosomal content. Many of the lymphoid cells interacted physically with each other (Fig 6) or with the epithelial cells, displaying membrane contact sites, in which adjacent cell membranes lay close to each other (<20 nm; Fig 7). Sometimes the lymphocytes formed knoblike, cytoplasmic protrusions that inserted deep into the cell coat of adjacent epithelial cells. At these projections the adjacent cell membranes often displayed contact sites with densification of the plasmalemma, as well as open clear vesicles in the epithelial cell cytosol facing the lymphocytic protrusion. Some of these epithelial cells were electron-lucent and in a state of degeneration (Fig 8).

In these areas the epithelial cells exhibited peculiar structural features, with development of awkward kinocilia on the luminal surface. Some atypical kinocilia projected out through deep cell membrane invaginations toward the luminal space. These cilia had a 9 + 0 microtubular arrangement and occurred more frequently than is usual for kinocilia (Fig 9). The apical cell membrane was more irregular, with cytoplasmic projections or knobs that seldom seemed to

Fig 7. TEM image showing physical interaction between lymphocyte and epithelial cell (original ×12,200). Both cells have prominent nucleoli. Note area of membrane specializations (arrows). Ly — lymphoid cell. Inset) Original ×77,000. Bar — 0.25 µm.



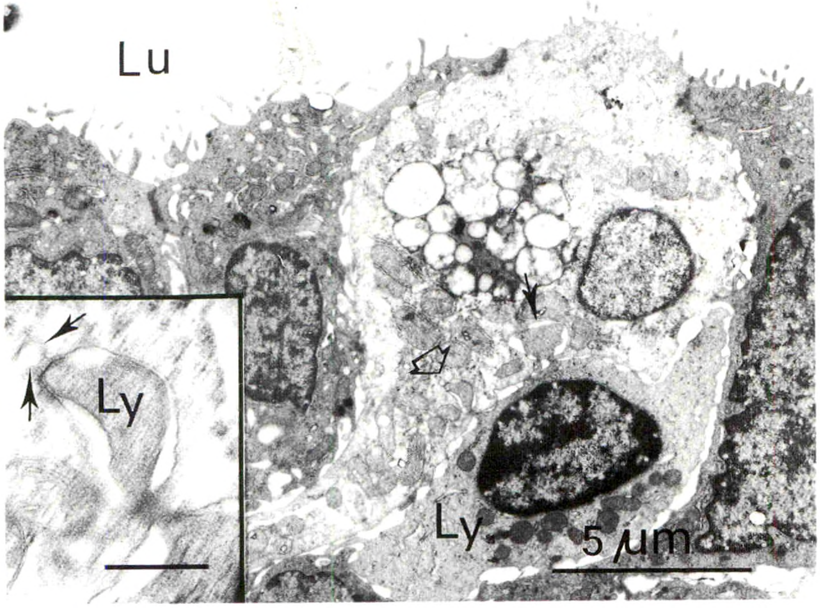


Fig 8. TEM image of partly vacuolated degenerating epithelial cell (original ×7,200). Note adherent large granular lymphocyte, showing cytoplasmic protrusions into epithelial cell (black arrows). Lu — lumen of ES, Ly — lymphoid cell. Inset) Lymphoid protrusion, marked with open arrow, at higher magnification (original ×37,500). Note contact site and associated small open vesicle in epithelial cytosol (arrows). Bar — 0.5 μm.

lose contact with the apical portion of the cell.

The cytoplasm of the abnormal cells typically contained rough endoplasmic reticulum in different stages of dilation, abundant in granular proteinaceous material suggesting a high protein synthesis. The cytosol contained many polyribosomes and monoribosomes, and occasionally, close to degenerated cells, ribosomelike structures (20 to 40 nm) occurred extracellularly. Well-developed Golgi structures were present, as well as bundles of fibrillar structures or filaments associated with the nucleus and nuclear invaginations. The cell nuclei frequently showed many prominent nucleoli, nuclear bodies, and peculiar chromatin patterns with infoldings that were not observed in the control material. Some cells contained membrane-bound vesicles in their apical region. The vesicle membrane sometimes coalesced with the apical plasmalemma and the contents had a texture similar to that of the intraluminal substance.

Focally, the basal lamina was thickened, irregular, or split up into several laminae, while in adjacent regions this structure appeared normal. In addition, there were irregularly shaped aggregations with the same electron density as the basal lamina associated with the serosal surface of the epithelial cell (Fig 10A). In some of these regions, plasma cells could be observed (Fig 10B). Similar material was seen in various amounts between individual epithelial cells.

*Subepithelial Space*. In the regions of increased epithelial mononuclear invasion, there seemed to be an increased deposition of collagen fibers and dense extracellular matrix. The cell coat of many fibrocytes showed numerous clathrin-coated vesicles and pits associated with deposited electron-dense material, indicating hypersecretion of extracellular matrix (Fig 11). Free cells such as lymphocytes, monocytes, and plasma cells were present. In other regions a normal, fairly well-vascularized loose connective tissue was observed. At the periphery, the border zone between bone and soft tissue was sometimes difficult to perceive on account of the deposition of partly calcified osteoid tissue. In the unmineralized zone, large conglomerates of calcospherites were frequently seen. Such calcospherites were also observed in the control material, but to a lesser extent, and there they were more restricted to the bone–connective tissue junction. Several psammoma bodies of various sizes were seen, sometimes close to the epithelium.

The number of blood vessels and the general vascular supply to the ES did not seem to be diminished as compared with the controls. The blood vessels, however, were sometimes abnormal, with signs of vasculitis. The basal lamina of the capillaries was thickened and multiplied into several distorted and fragmented laminae, and the vessels were often surrounded by a thick electron-dense layer that often included the pericytic cells (Fig 12A,B). The endothelial cells were often swollen, and sometimes, abnormal kinocilialike structures were seen, similar to those observed in the epithelium. Many blood vessels were obliterated by fibrin and cell degeneration products (Fig 12B). Some blood vessels appeared to be enwrapped in or surrounded by large lymphocytes and mononuclear monocyticlike cells that are not normally observed.

## DISCUSSION

The presumed role of the ED and ES in the pathogenesis of endolymphatic hydrops and MD rests on the assumption that these structures play a major part

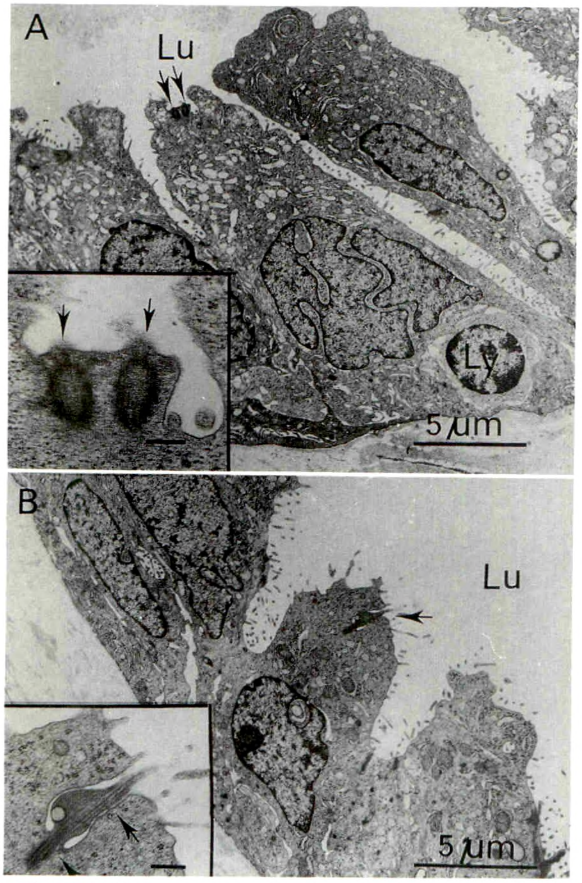


Fig 9. TEM images. Lu — lumen of ES. A) Epithelial cells showing interaction between small lymphocyte (Ly) and greatly disturbed epithelial cell (original ×5,400). Note development of awkward kinocilialike structures apically (arrows), together with organellar and nuclear aberrations. Inset) Original ×38,200. Bar — 0.25 µm. B) Epithelial cell with pathologically developed kinocilialike structure (arrows; original ×6,000). Inset) Original ×17,000. Bar — 0.5 µm.

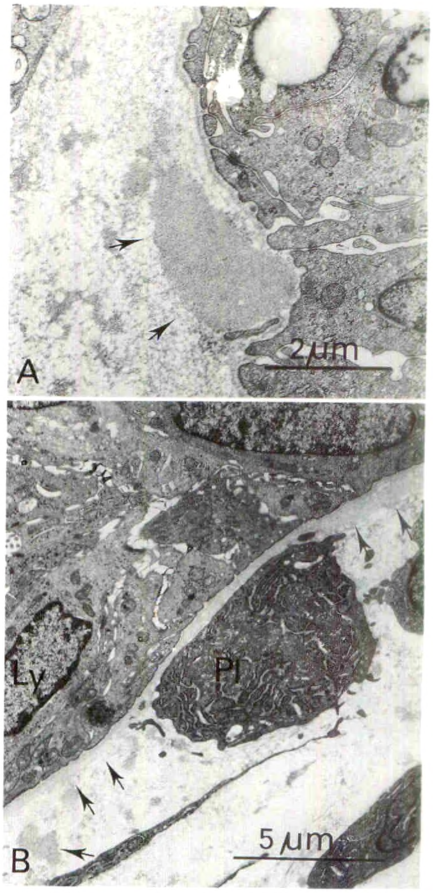


Fig 10. TEM images presenting basal aspect of epithelial lining. A) In close association with basal lamina, there are densely staining deposits (arrows; original ×17,200). B) In close association with thickened basal lamina and granular deposits (arrows), plasma cell (Pl) is seen (original ×7,800). Note intraepithelial lymphoid cell (Ly).

in the regulation of endolymph fluid homeostasis and endolymph resorption.[1-3] In addition, in MD, histopathologic disturbances in the structure of the ES have been reported.[2,4-7] The latter findings, however, are still controversial.

Studies in experimental animals have indicated that the ES might have an immunologic function.[10-12] Furthermore, immunologic challenge to this region has been found to give rise to reversible endolymphatic hydrops and cochleovestibular disturbances.[13,14] Studies on extraosseous ES specimens from an autopsy material and biopsy specimens from patients with acoustic neuroma showed the presence of at least two subsets of T lymphocytes both intraepithelially and subepithelially, suggesting an immune surveillance residing in the ES.[15,16] An ultrastructural study of biopsy material from a patient with presumed MD showed signs of epithelial hypersecretion of macromolecular substances into the ES lumen and an increased number of intraepithelial lymphocytes, with changes in the epithelial cell structure.[17] This latter biopsy material was more sparse, however, than that in the present study and did not allow a detailed examination of pathologic changes or of the morphology at the different levels of the intraosseous ES. The possibility of immunopathologic mechanisms in the development of MD has been pointed out by other authors.[18]

The results of the present study, which is the first systematic analysis of a complete vestibular aqueduct and intraosseous ES in a well-documented case of active MD, seem to provide evidence of involvement of immunopathologic mechanisms in the ES of this specific patient. Focal lymphocytic infiltration into, adherence to, and protrusions into disturbed and degenerating epithelial cells could be indicative of a T cell–mediated cytotoxic cell reaction targeting the epithelial cells of the ES. These epithelial cell changes might conceivably be the result of an autoaggressive immunologic reaction by the mononuclear cells. It is also possible that the epithelial cells had been subjected to a microbial invasion leading to a change in

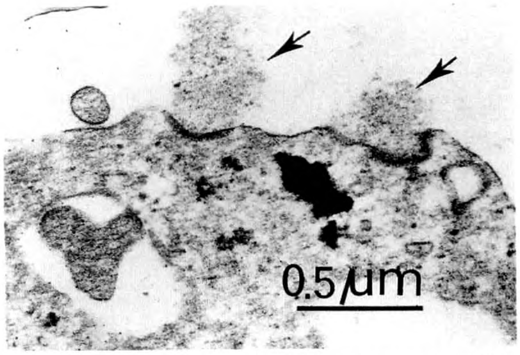


Fig 11. TEM image of pathologic secretory process from coated pits in fibrocytes into perisaccular tissue (original ×42,000). Secretory product (arrows) appears floccular and electron-dense.

antigenic properties that would attract a self-defending immune system, aiming at destroying infected cells. A third possibility could be an ES reaction caused by the ultrasound surgery. Such surgery represents thermal and mechanical trauma and could theoretically create an increased amount of debris in the ES due to degenerating cells in the inner ear. It could also result in increased exposure of inner ear antigen to the systemic circulation, leading to abnormal cell reactions in the ES. This seems a less plausible explanation, however, since the ultrasound procedure dated 2 years back, and would have affected the ES only indirectly; further, such trauma has not been known to influence or trigger immune mechanisms against irradiated tissue components.

The significance of the observed formation of abnormal multiple kinocilia in individual ES cells in the present specimen is unknown. It may represent a reversion to a more primitive stage as a response to altered microenvironmental factors. One rudimentary kinocilium is normally present in ES epithelial cells. Development of multiple kinocilia has been observed histopathologically in renal tubular epithelium in glomerular nephritis[19] and lupus nephritis,[20] and also in conditions leading to altered tonicity of renal fluids.[20]

Since the ES lies close to infection-prone regions of the middle ear, it might play a significant immunologic role in the defense of the inner ear against invading microorganisms. The ES may be exposed to antigen that is brought through the longitudinal flow of endolymph and attacks individual epithelial cells. Environmental provocation that is claimed to trigger autoimmune disease includes infections with bacteria or viruses,[21] although it seems generally accepted that the pathogenesis is a complex multifactorial interaction that is also linked to a genetic predisposition. Birgerson et al[22] have provided evidence of heredity for MD in certain families with a penetrance of 14%, and studies of the genome in MD patients have shown that they have an overrepresentation of HLA-DR2 antigen compared with controls.[23]

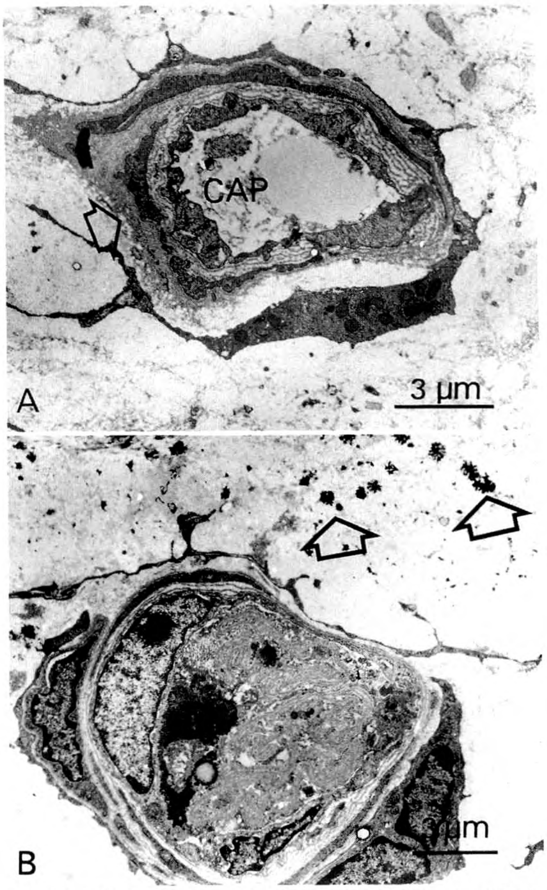


Fig 12. TEM images. A) Perisaccular capillary (CAP; original ×8,600). Note irregular multiple basal lamina. Electron-dense precipitate is also seen at abluminal aspect of vessel (arrow). B) Obliterated blood vessel close to bony wall of vestibular aqueduct (original ×7,500). Many small aggregations of calcospherites are seen (arrows).

The results of the ultrastructural analysis concerning the lymphocyte reaction indicated that many lymphocytes could have been cytotoxic T lymphocytes or natural killer cells that nonspecifically or possibly through antigen-specific adhesions had attached to the surface of epithelial cells and in some cases subsequently destroyed them. That such an infiltration of lymphoid cells has also been observed in autoimmune thyroiditis,[24] interstitial nephritis,[25] diabetes type I,[26] and systemic lupus erythematosus[27] could indicate that there was an autoimmune pathogenesis in this case of MD. The population of intraepithelial lymphoid cells seemed to include not only lymphocytes of various sizes, mostly medium-sized and large, but also monocytes and macrophages. A typical feature of the latter cells were the multivesicular bodies, which are known to be rich in acid phosphatase and are therefore considered to be a variety of lysosome.[28] These cells also showed differentiation and rough cell coats, indicating their phagocytic nature. No clear residual bodies or phagocytic material could be identified inside these cells, however, making it difficult to establish their nature and phagocytic target.

A striking feature in our specimen were the focal pathologic changes that frequently occurred in the epithelial basal lamina. These changes were also similar to the ultrastructural alterations found in conditions of autoimmune disease.[24-27] The nature of the thickening and multiplication of the basal lamina was unknown, but the ultrastructural appearance resembled that of basal lamina matrix, which could indicate an increased or pathologically altered intraepithelial production of such matrix. The presence of plasma cells subepithelially, often in close association with the thickened basal lamina, could indicate that these deposits also represent immune complexes. A deposit of such complexes has been observed previously in ES biopsy specimens from MD patients.[18] In that study, however, immune deposits were not found subepithelially, but in the luminal space and in a capillary wall. Lodging of such complexes in autoimmune disease can lead to serious manifestations of disease.[24-27] The basal lamina may act as a filter barrier during transepithelial flux of water and solutes, and an increase in thickness, multiplication, or other disturbances of its structure could lead to increased resistance and reduced endolymph resorption.

An abnormal basal lamina configuration was also present in perisaccular vessels in the immunologically affected ES regions. The multiple basal laminae formation, dense perivascular deposits, and sometimes obliterated vascular lumina were indicative of vasculitis. These findings were consistent with vascular histopathologic changes also present in other immune disease conditions, eg, interstitial nephritis[25] and systemic lupus erythematosus.[27] The perivascular deposits could, as earlier described in ES biopsy specimens from MD patients,[18] be immune complexes located on the capillary wall.

It has been reported that in human interstitial nephritis, fibroblasts proliferated[29] and increased their production of extracellular matrix, including collagen,[30] under the influence of cytokines. These cytokines are synthesized and secreted in increased amounts by immunoactivated T lymphocytes. A similar mechanism could account for the subepithelial aggregates of dense material and the signs of increased production of extracellular matrix in fibroblasts observed in the present ES specimen. In earlier reports on the ES in MD, perisaccular fibrosis was pointed out as one important feature.[2,4]

A reduction in size of the ES and vestibular aqueduct has been noted in patients with MD.[5] The numerous calcospherites, which are known to indicate an early stage of calcification,[31] and the new bone formation noted in the peripheral zone close to the bony surface, could suggest on ongoing process in the bony canal leading to bone apposition and narrowing of the vestibular aqueduct.

An observation not previously described in the ES either of normal individuals or MD patients was the presence of the filamentous structures showing some resemblances to stereocilia. Selective loss of short and middle stereocilia in the second and third rows of outer hair cells in the three upper cochlear turns has been observed in animals with induced experimental hydrops, and also correlated with hearing loss.[32] In a scanning electron microscopic study of a human inner ear from a patient with MD, loss of cilia was noted primarily in the basal turn of the cochlea and in the saccular epithelium.[33] It seems plausible that cilia from degenerating hair cells could be transported into the ES through the longitudinal endolymph flow. Ultrasound has been shown to produce degenerative changes in crista epithelium of the semicircular canals in the guinea pig.[34] It seems unlikely, however, that the stereocilia in our specific case would have been brought into ES tubules after the ultrasound procedure and not have been resorbed during the subsequent 2-year interval.

The results of the present study showed that the ES was histopathologically altered in many regions. In other regions, however, the ES structure appeared normal as compared with control material. In autoimmune-triggered type I (insulin-dependent) diabetes mellitus, morphologic studies of the pancreas have shown similar features, with areas of insulitis close to areas of normal islets. These different populations of islets probably represented a continuum of changes from normal to "diabetic" islets, and were correlated with the progression of the disease.[26] Thus, the focal ultrastructural histopathologic changes seen in the present study may be a manifestation of a slow progression of disease. It is also possible that different ES regions were selectively challenged both antigenically and by immunologic targeting.

The findings in this case of refractory MD suggested that a focal immunologic, autoaggressive, and tissue-damaging cell reaction, targeting the epithelial cells of the ES — a "sacitis" — was taking place. This may have resulted in a disturbance of its resorptive and/or fluid pressure regulatory functions. The ultrastructural findings in the present specimen indicated the possibility of increased resistance to fluid passage at the epithelial, subepithelial, and vascular levels. Further analysis of the ES in active and well-documented MD is required before firm conclusions can be drawn as to whether these changes can be linked to the development of the disease. The present study also emphasizes the need for time-consuming serial sectioning at all ES levels in the search for pathologic changes, as these changes may be focal.

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*Ann Otol Rhinol Laryngol* 106:1997

# ROLE OF ULTRASOUND IN THE ASSESSMENT OF VOCAL CORD FUNCTION IN INFANTS AND CHILDREN

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The determination of the cause of stridor in the infant and child is an important directive for the otolaryngologist. The limitations of current clinical diagnostic techniques have provided the impetus for pursuing newer, more practical techniques to diagnose vocal cord paralysis in children. The purpose of this paper is to describe a new technique to image the larynx and determine its role in discerning vocal cord paralysis in children. This study began with an animal study (lamb model) demonstrating that computerized ultrasound can accurately delineate laryngeal anatomy and function. The human protocol included 27 patients (age 1 day to 14 years): 15 with normal larynges and 12 with vocal cord paralysis. Recorded ultrasound studies of these subjects were compiled in a randomly ordered videotape and presented twice and viewed in a blinded manner by four expert raters. Statistical analysis of their readings revealed that ultrasound is a highly accurate technique to document vocal cord paralysis, with a high degree of interrater and intrarater reliability. Beyond the accuracy and consistency of computerized ultrasound, there are many other aspects of this technique that make it clinically attractive. Laryngeal ultrasound is noninvasive, painless, and widely accepted. It is relatively safe, with no radiation exposure and no need for sedation or anesthesia. It is well tolerated by patients and their families. The results are easily displayed and recorded and available for hard copy storage. This investigation should not be interpreted as an endorsement for laryngeal ultrasound as a replacement for endoscopy in children. This work does indicate, however, that ultrasound is a technique that can accurately address the special issue of vocal cord mobility in infants and children. At present, this is the primary use for laryngeal ultrasound, although with additional investigation and sophistication it is likely that ultrasound of the larynx may become useful for other purposes.

KEY WORDS — child, infant, stridor, ultrasound, vocal cord paralysis.

## INTRODUCTION

Vocal cord paralysis is reported to be the second most common cause of stridor in children.[1] The actual incidence of laryngeal paralysis, however, is difficult to assess and may be miscalculated due to the persistent difficulty in establishing the diagnosis.[2,3] Furthermore, the diagnosis of vocal cord paralysis in children appears to be increasing in frequency, possibly due to the increased survival of low–birth weight neonates with multiple congenital anomalies and to recent advances in instrumentation.[4]

While establishing the diagnosis of vocal cord paralysis in adults is usually straightforward, an examination of the larynx in infants and children may be problematic. The pediatric laryngeal examination may be complicated primarily due to the anatomic position and configuration of the pediatric airway and the difficulty in achieving adequate patient cooperation for a somewhat uncomfortable examination. These factors are compounded by the technical limitations of current equipment.

A wide variety of techniques to assess laryngeal function are available. These techniques can generally be divided into clinical and investigational categories. A practical technique optimally should be safe, noninvasive, well-tolerated, and reliable. The results should be reproducible, accurate, and recordable for future reference. Furthermore, the format of a good test of pediatric laryngeal function should be technically easy to perform and interpret, as well as relatively available for general use.

It is beyond the scope of this paper to extensively review all aspects of each clinical or research tool available; however, specific technical limitations for the use of certain diagnostic studies in infants and children will be delineated.

## CLINICAL TECHNIQUES

*Direct Visualization.* Most children under the age of 6 cannot tolerate indirect laryngoscopy, so direct laryngoscopy under general anesthesia is used with great frequency in this age group. Direct laryngoscopy

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From the Department of Pediatric Otolaryngology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Harvard University.

This work was submitted and accepted as a candidate's thesis for the American Laryngologic, Rhinologic and Otologic Society, 1991. It was presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, April 13-14, 1992, and portions of it were published in the 1992 *Transactions of the American Broncho-Esophagological Association.*

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offers the major advantage of making possible accurate visualization of the actual anatomy. However, there are technical pitfalls associated with this technique. One must be careful when inserting the laryngoscope in the introitus of the larynx, because the position of the tip of the metal laryngoscope may immobilize the true vocal cord, thereby erroneously giving the impression of vocal cord paralysis.[5,6] The need for general anesthesia may alter the natural dynamic function of the larynx. Even without pharmacologic paralysis, the plane of anesthesia may significantly alter vocal cord mobility. A deep plane will diminish ventilatory drive and thereby vocal cord excursion. A lighter plane of anesthesia will lead to laryngospasm, which may be a life-threatening event. Unfortunately, there is only a very narrow window in the plane of anesthesia when it is light enough for the patient to demonstrate truly representative vocal cord activity while deep enough to avoid laryngospasm. The ability to perform a safe laryngeal examination of adequate duration to be assured of vocal cord function under general anesthesia is highly dependent on the degree of experience, skill, and patience of the participating anesthesiologist. It is not uncommon for this portion of direct laryngoscopy to be brief, unsatisfactory, or aborted completely due to concern regarding patient safety.

In the 1970s, flexible laryngoscopy became available. With the use of fiber optics and improved instrumentation, visualization of the infant larynx was enhanced.[7,8] This technique has a variety of favorable characteristics, including that it can be performed without anesthesia, allowing for a direct view of the larynx in the normal physiologic state, unencumbered by pharmacologic agents. Furthermore, the flexible laryngoscope is a relatively inexpensive piece of equipment that is widely available. This technique has become the gold standard for examining laryngeal dynamics.

The successful use of the flexible laryngoscope is, however, somewhat limited by the expertise of the endoscopist and by certain anatomic factors, including the size of the nares, the patency of the choanae, the configuration of the infant epiglottis, and the higher, more anterior position of the pediatric larynx. Grundfast and Harley[9] note the increased risk of complications when flexible endoscopy is performed on patients with the following problems: significant airway obstruction, cardiac anomalies, history of cardiac arrhythmia, or coagulopathy.

Further limitations with the flexible endoscope exist. The 3.4-mm scope, currently the most commonly utilized flexible laryngoscope, has a very small field of view. The tip of the laryngoscope rotates only 180° and at times is difficult to position to obtain an adequate view of the complete endolarynx. There is no portal for suction in the pediatric flexible laryngoscope, so secretions cannot be removed. Many children, especially those with neurologic disorders, have pooling of secretions, and the lack of suction may severely limit visualization of the anatomy. Less frequently, nasal trauma, vomiting, or the induction of a vagal reaction such as bradycardia or laryngospasm may occur.[10]

By far the most limiting factor in the use of the flexible laryngoscope in children is the degree of patient cooperation necessary to insert the flexible tube in the nose of the young child. This barrier is frequently difficult to overcome. In many cases, the flexible scope is tolerated for a very brief period of time, affording only a fleeting glimpse of the larynx that might be inadequate to assess vocal cord function.[11] The addition of recording or video equipment to flexible endoscopy will significantly aid the otolaryngologist by allowing a more leisurely review of the endoscopic examination, although even on review, the rapidly moving structures may be difficult to interpret. Furthermore, many offices and hospitals do not have the expensive equipment necessary for video documentation. Personal experience has shown that in approximately 15% to 20% of pediatric patients, particularly those less than 3 years old, the endolarynx cannot be adequately visualized by this method.

*Imaging Techniques.* Williams et al[12] have described several radiographic findings apparent on plain films consistent with vocal cord paralysis, including distention of the hypopharynx, underdistention of the trachea, and overdistention of the laryngeal ventricle during inspiration. These authors acknowledge that the radiographic findings may be subtle and since they are general secondary signs of airway obstruction, they are not specific for the diagnosis of vocal cord paralysis. Furthermore, the role of anterior, posterior, and lateral neck films to detect vocal cord paralysis in infants and children is limited, since these films are difficult to obtain and interpret in infants.

Videofluoroscopy can be helpful, although the results may be difficult to interpret in very small children.[13] In this technique, the use of a videocassette recorder is essential to allow for repeated review of the examination to enhance the ability for adequate interpretation. The major drawback to this technique is the degree of difficulty in demonstrating and interpreting a vocal cord paralysis in the infant and young child.

Laryngography is a fairly invasive radiographic technique in that it involves the introduction of a contrast agent into the airway. Premedication is often necessary to successfully accomplish the examination. This technique has been shown to be the most sensitive to demonstrate even subtle mucosal changes of the vocal cords. Significant medical complications such as reactive mucosal edema, adverse pulmonary reaction, and respiratory arrest have restricted the role of contrast laryngography.[14]

Ma[15] also described radiographic signs of vocal cord paralysis, including vocal cord atrophy, failure of abduction of the vocal cord, and immobility of the pyriform sinus. He used laryngeal tomography and positive contrast laryngograms to demonstrate these findings. The techniques described in Ma's paper require fast, deep inspirations or other voluntary maneuvers to enhance visualization of vocal cord dysfunction. Infants and children often are not able to comply with these directions.

Noyek et al[16] have also relied on contrast laryngography and laryngeal tomography to demonstrate vocal cord paralysis. With these techniques, they note incomplete abduction of the involved vocal cord, dilation of the ipsilateral ventricle, a flattened subglottic arch, a widened pyriform sinus on the paralyzed side, and a flattened false vocal cord and aryepiglottic fold on the involved side. Further observations included that on tomography the paralyzed vocal cord appeared to be thinner and below the level of the normal vocal cord during quiet respiration and above the normal vocal cord during phonation. Laryngeal tomography and laryngograms are considered obsolete, due not only to the degree of radiation exposure and inherent risk to the pediatric patient, but also to the existence of preferable advanced technology.

Initially, computed tomography (CT) had been noted to be less sensitive than contrast laryngography to diagnose vocal cord paralysis. Agha[17] examined 8 patients with laryngeal paralysis and compared laryngoscopy (both direct and indirect) to contrast laryngography and laryngeal CT. Fifty percent of the patients on CT demonstrated nonspecific and inconclusive findings. Based on the findings of 4 patients, the author summarized the CT appearance of vocal cord paralysis as an abnormal arytenoid cartilage position more anterior with anteromedial tilting on the paralyzed side, a dilated pyriform sinus on the paralyzed side, tilting of the thyroid cartilage, and dilation of the laryngeal ventricle on the paralyzed side. Agha felt that the CT findings were subtle and require careful analysis, while laryngography demonstrated more obvious, distinct diagnostic findings. He further noted that technical advances such as thinner cuts and more rapid CT imaging would improve the likelihood that CT could accurately diagnose vocal cord paralysis. With more advanced CT scanning, using rapid scanning CT, Mancuso et al[18] noted that CT scanning was equal to or better than contrast laryngography in 90% of cases.

Newer imaging techniques have also been evaluated for use in the laryngeal examination. Cine-computed tomography (cine-CT), proposed by Frey et al,[13] is capable of imaging up to eight levels in 224 milliseconds. Their series included patients with a variety of airway lesions; only 2 had vocal cord paralysis. Cine-CT correctly identified both of these patients as having abnormal vocal cord function, although in 1 patient with bilateral vocal cord paralysis, the cine-CT findings suggested unilateral vocal cord paralysis. Although preliminary work with cine-CT is compelling, there are several drawbacks to this technique. Currently, this technology is not available in most hospitals. Sedation is frequently necessary, thereby increasing morbidity, as well as possibly altering the normal physiologic respiratory patterns. Furthermore, cine-CT exposes the patient to radiation hazard, is not portable, and requires a lengthy examination.

*Perceptual Acoustic Analysis*. Perceptual acoustic analysis involves the assessment of quality, pitch, loudness, and flexibility of the voice. Studies as early as the 1830s investigated the variation in human voice and crying.[19] Flatau and Gutzmann[20] (1906) were the first to describe the pitch and phonetic characteristics of newborn babies' cries. Several authors have reported the ability to distinguish various causes of cry, ie, hunger versus pain; however, to assess more sophisticated information on a perceptual basis requires a considerable amount of clinical experience. Aronson[21] has delineated a systematic approach to the perceptual examination of the patient with a clinical voice disorder. He notes the ability to audibly distinguish a patient with a brain stem lesion at the level of the 10th cranial nerve from a patient with a brain stem lesion at a higher level. Hirschberg and Szende[22] have also categorized pathologic voices. Although much of their work relied on spectrographic analysis of tape-recorded vocalization, they have produced an extensive classification of perceptual differences among various pathologic cries, stridor, and cough, which are audible and discernable with the human ear alone. The ability to discern such variations in cry is an extraordinary clinical attribute, and perceptual interpretation is certainly subjective and difficult to quantify. These considerations significantly limit the widespread use of perceptual acoustic analysis for diagnostic purposes.

## INVESTIGATIONAL TECHNIQUES

A wide variety of investigational techniques exists for the assessment of laryngeal function. A partial list includes computerized acoustics analysis; electrodiagnostic methods, including electromyograms, neuromyography, and reflex myography; laryngeal function tests, including ultra-high-speed cinematography, stroboscopy, and glottography; and dynamic studies.[23-34] These techniques are, for the most part, currently research tools. While most of them will, in the broad sense, identify vocal cord paralysis, for the most part, they are aimed at obtaining more sophisticated quantitative information about vocal fold activity. Therefore, if these techniques were available for clinical use in the pediatric population, they would provide significantly more information than the presence or absence of vocal cord mobility.

Unfortunately, most of these techniques are not currently available for clinical use in the neonate, infant, or child. There are specific limitations inherent in the testing procedures, apparatus, or level of technology that restrict their role in addressing the clinically relevant question of vocal cord mobility in the infant and child. Specifically, computerized acoustic analysis is limited in pediatrics due to the inability of this patient population to be able to voluntarily comply with repetitive vocal production.[35] Methods of electrodiagnosis of laryngeal function in children have been limited by the lack of normative data in this age group, as well as the lack of necessary smaller (pediatric-size) instrumentation.[36] The degree of patient cooperation needed to complete these tests, as well as the technical difficulties with electrode technology and placement in the pediatric patient, confounds successful use of electrodiagnostic methods.[37]

Laryngeal function studies rely greatly on equipment that is extremely expensive and not readily accessible. Additional technical difficulties include poor pediatric patient tolerance of the procedure, patient inability to perform specific maneuvers on command, and the degree of complexity of the interpretation of the results, requiring highly trained and specialized assistance.[38]

## ULTRASOUND

As a practical clinical test has to be safe, noninvasive, well tolerated, technically easy to perform and interpret, and generally available, the current investigational techniques, while exciting and informative, are not practical for clinical application in the pediatric patient. Clinical application of ultrasound to the laryngeal examination was approached in the early 1970s by a variety of investigators.[39,40] This work was significantly limited by the technology available at the time, which produced a tracing or ultrasonic glottogram of the vibratory patterns of the vocal cords, in contrast to the current availability of computerized ultrasonography to image the actual anatomic structures. In spite of the limitations, the early investigators recognized several advantages of ultrasound, namely, the ability to record vibratory action continuously, without interference from the tongue or mandibular movement.[41] They were impressed with the simplicity, relative safety, and comfort of the study technique. Although the investigators acknowledged the constraints of the level of ultrasound technology available at that time, each foresaw the promise of the medical application of this technique.

Even after significant technological advances, laryngeal ultrasound was not widely pursued, due to the tenet that the air in the larynx and trachea was considered to be an absolute barrier to the ultrasonic beam and would reflect over 99% of the beam.[42] This led to the belief that the air-tissue interface present in the larynx would not permit reliable ultrasound transmission. Currently, most clinicians continue to believe that ultrasound requires a tissue-fluid interface, such as fetus and amniotic fluid or cardiac muscle and blood, to be effective. However, in 1987, Raghavendra et al[43] described the incidental identification of the larynx while performing computerized ultrasound scans of the thyroid gland. Stimulated by this finding and dissatisfied by the current state of clinical examination of the pediatric larynx, we began the current investigation.

Computerized ultrasonography has significantly enhanced three important parameters of image quality, namely, detail resolution, contrast resolution, and uniformity throughout the field of view.[44] These advances have decidedly increased diagnostic confidence associated with ultrasonography.

## METHODS AND MATERIALS

Initial animal experiments allowed for the comparison of the actual anatomy with the ultrasound image. This was achieved by "labeling" the corniculate process of the arytenoids with submucosal injections of water. These blebs served as hypoechoic markers that were easily identified on the ultrasound image, documenting the level of the arytenoid cartilages. Additional animal experimentation allowed laryngeal ultrasound to be performed following unilateral surgical resection of the recurrent laryngeal nerve. The creation of a known iatrogenic lesion

TABLE 1. DEMOGRAPHICS OF SUBJECTS

| | *Study Group**<br>*(n = 12)* | *Control Group*<br>*(n = 15)* |
|---|---|---|
| Male to female ratio | 4:8 | 5:10 |
| Age range | 1 mo to 14 y | 1 d to 8 y |

*Vocal cord paralysis (2 right-sided, 10 left-sided) caused by surgery in 7 cases (5 for cardiac problems, 2 other) and by neurologic problems in 5 cases.

allowed for interpretation of the subsequent ultrasound with assurance of the specific underlying physiologic abnormality. Following a pilot study that included five lambs, data were gathered that were suggestive of the ability of ultrasound to adequately visualize the laryngeal anatomy and identify vocal cord paralysis. This information encouraged us to pursue a protocol to establish the role of ultrasound in human neonates and children.

A protocol was developed to gather patients with vocal cord paralysis of known and unknown causes and a similar age-matched cohort of children with normal vocal cord mobility to evaluate the ability of ultrasound to identify vocal cord function.

Fifteen normal children who were undergoing an unrelated otolaryngological procedure (myringotomy and tube placement) were solicited to voluntarily participate in the study as the control group. An informed consent was obtained from each patient's family. Each subject underwent two laryngeal examinations: one by flexible endoscopy, which served as the gold standard, and the other by ultrasound. In preparation for the nasal introduction of the flexible endoscope, three drops of 1/4% Neo-Synephrine and 4% lidocaine hydrochloride were inserted into the right and left nostrils. Flexible endoscopy was performed with the 3.4-mm Olympus flexible laryngoscope and the xenon light source. Studies were recorded with the Sony VHS videocassette recorder and 0.5-in Scotch videotape. Ultrasound examination was performed in the supine position in infants and the sitting position in older children, with the Acuson 128 and a 7-MHz transducer.

The average duration of the flexible endoscopy examination was 7 to 10 minutes, and the average duration of the ultrasound examination was less than 5 minutes. Twelve patients with unilateral vocal cord paralysis were examined in similar fashion with flexible endoscopy findings as the gold standard, except in 3 patients. In these 3 patients, flexible endoscopy was not adequate due to either choanal atresia, excessive secretions, or anxiety. In these cases rigid, direct laryngoscopy was performed.

The videotaped evaluations included those of 16 boys and 11 girls who ranged in age from 1 day to 14 years. There were 15 patients with normal vocal cord function and 12 patients with vocal cord paralysis. Of the patients with vocal cord paralysis, 10 paralyses were left-sided and 2 were right-sided. Data concerning the study group, including the causes of the vocal cord paralysis, are listed in Table 1. All videotaped segments of the laryngeal ultrasound studies were uniformly 60 seconds in duration. All 27 videotaped laryngeal ultrasound segments were presented twice, randomly within the tape so that intrarater reliability could be determined. A composite tape of the original studies and the retest segments was formed in an order created by a computer-generated list of random numbers.

All aspects of the animal and human studies were reviewed and approved by the institutional Human Investigational Committee, and all ethical considerations were met with satisfaction.

*Raters.* Four ultrasonographers were solicited to participate in the human study as raters. These raters were pediatric ultrasonographers with between 4 and 14 years of experience. The viewing time required for the compiled videotape was approximately 2 hours. The raters had the disadvantage of not personally performing the studies, not being aware of the clinical history or the patient's state of respiration (quiet, crying, or phonation), and having only 60 seconds of a study to review. The data from the returned forms were evaluated statistically in order to determine subjective confidence level, with verification of interrater and intrarater reliability.

*Training.* Prior to judging of the ultrasound studies, a 6-minute training tape introduced the topic to the raters. Examples of normal and paralyzed larynges on endoscopy and ultrasonography were shown with and without anatomic labels. Raters were instructed to view the tape sequentially. Each segment could be reviewed as often as the individual rater desired. The raters were instructed to view the tape alone and did not receive any feedback or additional instruction during the training segment of the videotape. The rating forms were directed at identifying right versus left vocal cord function, which was quantified as normal, abnormal with less motion, and abnormal with no motion.

## RESULTS

*Sonoanatomy of Larynx.* The experience gathered through this study has helped to document and define the "sonoanatomy" of the larynx. Ultrasound structures are defined by the amount of echoes absorbed or reflected by body organs. Structures that do not reflect many echoes will appear black or dark, and are termed hypoechoic. Structures that reflect echoes

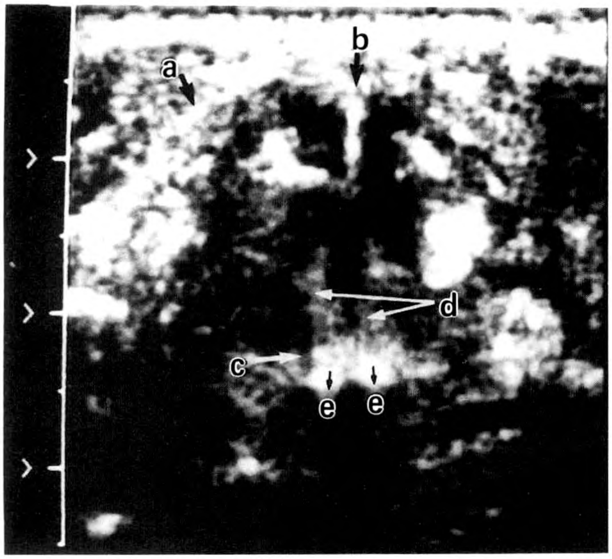

Fig 1. Ultrasound image of normal larynx. a — thyroid cartilage, b — anterior commissure, c — ventricle, d — true vocal cord, e — arytenoids.

appear white or light, and are termed hyperechoic. With ultrasonography, the true vocal cords are smooth, well-defined, paired structures that are hypoechoic. The false vocal cords appear hyperechoic. These differences may represent the different amount of muscle in the true vocal cords versus the false vocal cords, with muscle being hypoechoic. The arytenoids are prominently visualized as round, echogenic structures that are readily identified due to their anatomic location, as well as their obvious in and out movement on abduction and adduction. The larynx is encased by the thyroid cartilage, a boomerang-shaped structure of middle echogenicity. The rima glottidis, the lumen between the vocal cords, is hypoechoic and triangular-shaped (Fig 1). Three representative still pictures of the ultrasound scan have been selected to demonstrate the differences in appearance on inspiration and expiration of the larynx during abduction and adduction; however, the dynamic ultrasound appearance of the normal and paralyzed larynx is best appreciated when viewed on real-time videotape. In the normal larynx, the vocal cords move in a symmetric manner during the respiratory cycle (Fig 2). In the paralyzed larynx (Fig 3), several differences are apparent. These are 1) asymmetry of motion, 2) abnormal arytenoid movement, 3) a shorter, lower position of the paralyzed vocal cord, and 4) anterior bowing of the flaccid vocal cord.

*Statistical Methods*. Each endoscopy study was summarized as normal, vocal cord paralysis on the right, or vocal cord paralysis on the left. The ultrasound evaluations were summarized in the same way, but also included the category "inadequate examination." In order to assess the role of ultrasound in the determination of vocal cord paralysis, we performed statistical tests to examine the reliability and reproducibility of the technique. The statistics that assess reliability and reproducibility are the κ[45] and the weighted κ.[46] The κ assesses agreement between a rater and a gold standard or between first and second readings by the same rater beyond that expected by chance alone. The weighted κ also assesses agreement, but penalizes disagreements that are worse than others. Both κ values range from 0 (no agreement) to 1 (complete agreement); values greater than .70 or .75 represent very good agreement beyond chance.

The weighted κ was used to examine agreement between each of the raters and the gold standard. A diagnosis of vocal cord paralysis in a normal study was penalized more heavily than diagnosing right-sided paralysis when it was, in fact, on the left. To examine the joint agreement between the four raters and the gold standard, the statistic G, which also evaluates agreement beyond chance, was calculated.[47]

Reproducibility was addressed by comparing test-retest results whereby raters in a blind and random

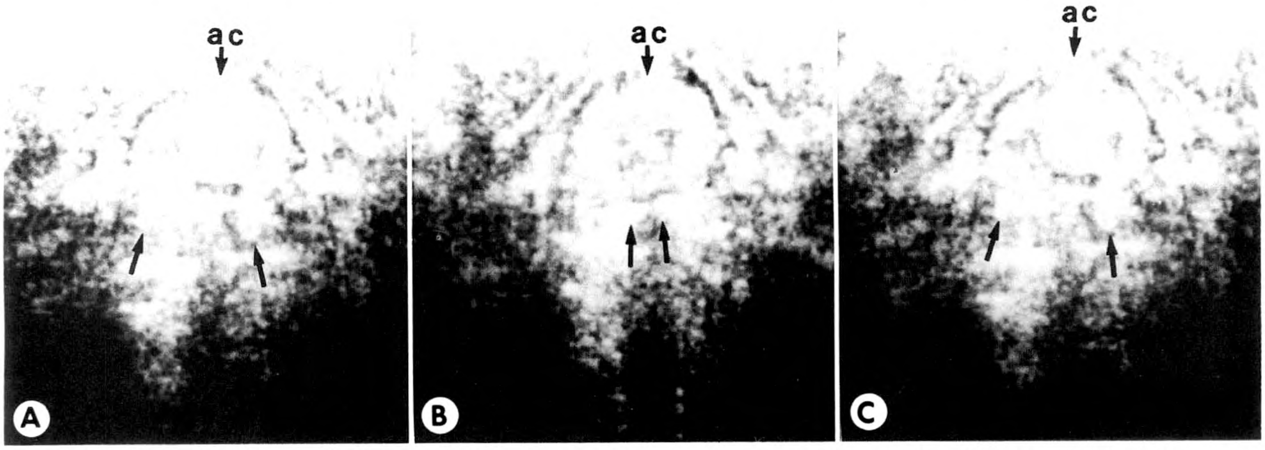

Fig 2. Normal larynx. Arrows — arytenoids, ac — anterior commissure. A) Abduction. B) Adduction. C) Abduction.

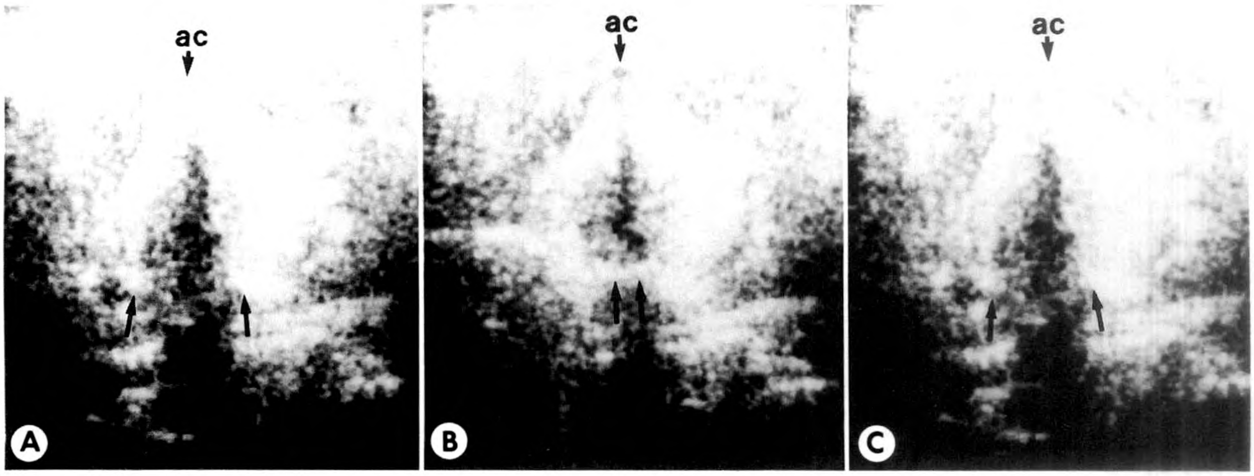

Fig 3. Left vocal cord paralysis. Arrows — arytenoids, ac — anterior commissure. A) Abduction. B) Adduction. C) Abduction.

fashion reread the same study. The κ statistic was employed to determine the ability of each rater to interpret the same study with consistency over time. A judgment could not be made on whether it was good or bad to have considered a study inadequate, so no weights were used.

*Reliability of Raters.* Reliability of the raters gives assurance that a new test will produce results that will compare favorably to the gold standard. The diagnoses made by each reader are cross-tabulated with the gold standard diagnosis in Table 2. Three of the four raters judged some studies to be inadequate, and those studies are not included in the calculation of the weighted κ statistic. The studies included in the calculation are those that were, in fact, rated, and the inadequate columns of the Table simply account for all the gold standard studies. Our weighted κ values range from .75 to .91, and all p values fall below .05 (.01 to .004), which indicates that our raters agree with the gold standard with high accuracy in a significantly nonrandom fashion. If we look at the diagonal of each gold standard by rater table — where there is agreement — then we see that the raters make the correct assessment from 87% to 94% of the time.

While this is a highly accurate rate, there remains a 6% to 13% failure rate. This rate primarily repre-

TABLE 2. AGREEMENT BETWEEN FOUR RATERS AND GOLD STANDARD

| *Rater* | *GS* | *Inadequate** | *Normal* | *L Abnormal* | *R Abnormal* | *Total for* $\kappa_w$ |
|---|---|---|---|---|---|---|
| Rater 1 | Normal | 5 | 25 | 0 | 0 | 25 |
| | L abnormal | 4 | 3 | 13 | 0 | 16 |
| | R abnormal | 2 | 2 | 0 | 0 | 2 |
| | Total studies | 11 | 30 | 13 | 0 | 43 |
| | | | $\kappa_w = 0.76$; $p = .01$; % correct = (25 + 13 + 0)/43 = 88% | | | |
| Rater 2 | Normal | 1 | 29 | 0 | 0 | 29 |
| | L abnormal | 2 | 2 | 15 | 1 | 18 |
| | R abnormal | 0 | 0 | 0 | 4 | 4 |
| | Total studies | 3 | 31 | 15 | 5 | 51 |
| | | | $\kappa_w = 0.91$; $p = .004$; % correct = (29 + 15 + 4)/51 = 94% | | | |
| Rater 3 | Normal | 0 | 30 | 0 | 0 | 30 |
| | L abnormal | 0 | 2 | 15 | 3 | 20 |
| | R abnormal | 0 | 2 | 0 | 2 | 4 |
| | Total studies | 0 | 34 | 15 | 5 | 54 |
| | | | $\kappa_w = 0.82$; $p = .007$; % correct = (30 + 15 + 2)/54 = 87% | | | |
| Rater 4 | Normal | 3 | 27 | 0 | 0 | 27 |
| | L abnormal | 3 | 4 | 13 | 0 | 17 |
| | R abnormal | 0 | 1 | 0 | 3 | 4 |
| | Total studies | 6 | 32 | 13 | 3 | 48 |
| | | | $\kappa_w = 0.79$; $p = .01$; % correct = (27 + 13 + 3)/48 = 90% | | | |

*Gold standard (GS) has no "inadequate study" category, so studies in that category cannot be included in calculation of weighted κ ($\kappa_w$). Inadequate category is included in Table to account for all 54 studies.

TABLE 3. AGREEMENT BETWEEN FIRST AND SECOND READINGS FOR FOUR RATERS

| | | *Second Reading* | | | | |
|---|---|---|---|---|---|---|
| | *First Reading* | *Inadequate* | *Normal* | *L Abnormal* | *R Abnormal* | *Total for* κ |
| Rater 1 | Inadequate | 3 | 1 | 0 | 0 | 4 |
| | Normal | 3 | 13 | 0 | 0 | 16 |
| | L abnormal | 1 | 0 | 6 | 0 | 7 |
| | R abnormal | 0 | 0 | 0 | 0 | 0 |
| | Total | 7 | 14 | 6 | 0 | 27 |
| | | κ = .69; p = .04; % agreement = (3 + 13 + 6 + 0)/27 = 81% | | | | |
| Rater 2 | Inadequate | 1 | 1 | 0 | 0 | 2 |
| | Normal | 0 | 15 | 0 | 0 | 15 |
| | L abnormal | 0 | 0 | 7 | 0 | 7 |
| | R abnormal | 0 | 0 | 1 | 2 | 3 |
| | Total | 1 | 16 | 8 | 2 | 27 |
| | | κ = .87; p = .009; % agreement = (1 + 15 + 7 + 2)/27 = 93% | | | | |
| Rater 3 | Inadequate | 0 | 0 | 0 | 0 | 0 |
| | Normal | 0 | 16 | 1 | 1 | 18 |
| | L abnormal | 0 | 0 | 7 | 0 | 7 |
| | R abnormal | 0 | 0 | 0 | 2 | 2 |
| | Total | 0 | 16 | 8 | 3 | 27 |
| | | κ = .86; p = .009; % agreement = (0 + 16 + 7 + 2)/27 = 93% | | | | |
| Rater 4 | Inadequate | 2 | 1 | 0 | 0 | 3 |
| | Normal | 1 | 14 | 0 | 0 | 15 |
| | L abnormal | 0 | 1 | 6 | 0 | 7 |
| | R abnormal | 0 | 1 | 0 | 1 | 2 |
| | Total | 3 | 17 | 6 | 1 | 27 |
| | | κ = .74; p = .02; % agreement = (2 + 14 + 6 + 1)/27 = 85% | | | | |

sents false negatives. In addition, there is some concern that a number of studies were considered inadequate by the raters. The percentage of studies judged inadequate was 20%, 6%, 0%, and 11% for raters 1 to 4, respectively. On the whole, however, inadequate studies were evenly distributed among those subjects judged normal and abnormal by endoscopy; this finding suggests that inadequacy of the examination was not related to the specific diagnosis and that the deletion of these examinations in the calculation of the κ does not introduce bias. Factors that may enhance diagnostic accuracy and reduce the number of studies that are inadequate include longer examinations (currently, each examination shown was only 60 seconds), access to clinical history, improved technical quality of the studies, and additional rater experience and confidence in the interpretation of laryngeal ultrasound.

In practice, if a rater were uncertain how to rate an ultrasound, additional raters might be called in. We looked at the joint agreement of all four of our raters with the gold standard, which in effect summarizes the agreement across all four raters in Table 2. Even higher reliability and accuracy are achieved when additional raters review an ultrasound ($G = 11.3$, $p \leq .001$).

The weighted κ statistic, the G statistic, and the percent correct figures indicate that the laryngeal ultrasound interpretations are reliable and highly accurate.

*Consistency of Raters.* Intrarater reliability and reproducibility demonstrate the desired consistency of interpretations of the same materials by the same rater at different times. The data for the analysis of test-retest comparison readings for each rater are shown in Table 3. The κ values are .69 or better and suggest a high degree of reproducibility. The test-retest readings reflect minor variability among the four raters as demonstrated by their individual κ values, but on the whole, each of the four raters was able to consistently interpret the study examinations.

Another area of concern when assessing a new clinical test is the degree of subjectivity in the interpretation of the results. It is most important to bear in mind that although even the gold standards (flexible endoscopy and direct laryngoscopy) also are open to perceptual error by the viewer, we wanted to be certain that the interpretability of ultrasound was not excessively idiosyncratic.

This issue is reflected by the minor variation in the number of accurate readings among the raters. Since all of the raters had a statistically high degree of accuracy, it appears that the amount of subjective perceptual variation is minimal and not of clinical concern. Of interest is the different number of studies judged to be inadequate by the individual raters (11 for rater 1, 3 for rater 2, none for rater 3, and 6 for rater 4). These differences may reflect differences among the raters, such as level of expertise, adaptability to interpreting a new technique, personal philosophy concerning making a diagnosis, or degree of comfort concerning decision-making. These numbers also are influenced by the technical limitations caused by the inclusion of suboptimal studies performed by an otolaryngologist rather than an ultrasonographer.

## DISCUSSION

The first reference to the use of ultrasound to demonstrate the vibrations of the vocal cords was by Mensch[48] in 1964. This early report was followed by work from Japan on extirpated larynges.[49] Kitamura et al[50] demonstrated echoes associated with phonation, which they termed a "vibration echo." At this time, the ultrasound technology produced an undulating tracing of the echo secondary to the vibration and movement of the vocal folds, which was called an ultrasonoglottogram. Ultrasonography relied on two transducers placed on both sides of the neck. The accurate alignment and placement of these transducers were critical to obtaining consistent information.[51] Over the ensuing years, there have been many advances in technology, including changes in transducer construction and the coupling of a specialized computer with ultrasound imaging.

Computerized ultrasonography was introduced in 1983. The computer software has specialized data processing capabilities that allow for the maximal extraction of diagnostic information. This technique enables one to demonstrate the actual anatomic structures of the larynx rather than a tracing on an oscilloscope. These improvements have significantly enhanced the image quality, detail resolution, and diagnostic accuracy associated with ultrasonography.[44]

Computerized ultrasonography is a noninvasive, painless technique that has widespread availability. There is no radiation exposure or hazard. There is no need for sedation or anesthesia to allow for an examination that is truly representative of unaltered physiologic laryngeal activity. Examination time is brief and it can be tolerated by and performed on neonates as well as older children. The findings are easily recorded for hard copy storage and for pretreatment and posttreatment comparisons, or for sequential comparative studies. The computerized ultrasound machine is an expensive unit; however, it is already currently available in most hospitals. Therefore, laryngeal ultrasound represents an additional use for preexisting equipment. The cost for each examination should be reasonable. Although no new technical personnel should be required to accomplish laryngeal ultrasonography, minimal training is necessary to obtain and interpret the studies. The apparatus is portable and can be used at the bedside, such as in the intensive care unit, as well as in the radiology suite.

Parental and patient acceptance is excellent, perhaps due to maternal familiarity with fetal ultrasound monitoring during pregnancy. Although no attempt was made in this study to quantify patient response to the ultrasound examination, several consistent observations were made. There was ready acceptance of the ultrasound examination, even though several patients refused to participate in the protocol or withdrew when they realized that the protocol included flexible endoscopy. Further, ultrasound was tolerated by a wide range of patients, as demonstrated in this study, even when several of the patients were unable to undergo successful flexible laryngoscopy due to either choanal atresia, excessive secretions, or extreme discomfort or fear. All of the patients tolerated laryngeal ultrasound without difficulty.

These inherent properties of ultrasound make it an attractive technique. The true value of ultrasound to accurately diagnose vocal cord paralysis in infants and children rests on its reliability and reproducibility compared to the gold standard. In this study, the gold standard was flexible endoscopy in 24 patients, and direct laryngoscopy in 3 patients. The $\kappa$ values reflect a high confidence level for reproducibility and accurate interpretation of the evaluation of the dynamic normal or paralyzed larynx. The confidence factors outlining intrarater and interrater reliability can be inferred from the large values of the weighted $\kappa$ and $\chi^2$ statistics. These statistical analyses imply that ultrasound is a highly accurate, reproducible, and reliable measure of vocal cord function.

This work establishes the following criteria to determine vocal cord paralysis with ultrasound: 1) asymmetry of motion, 2) abnormal arytenoid movement, 3) shorter, lower position of the paralyzed vocal cord, and 4) anterior bowing of the flaccid vocal cord.

If uncertainty persists following an initial ultrasound examination, this technique offers several additional advantages. First of all, due to the noninvasive nature of the study and the high degree of patient comfort and acceptance, it is expected that most patients would willingly comply with extended or repeat examinations. Furthermore, ultrasound has the built-in capacity to record all examinations, thereby facilitating video documentation for consultation with colleagues. We examined the benefit of having multiple raters view the studies. The impact of joint agreement reveals that multiple viewers will increase the accuracy of the ultrasound interpretation. The ability to record all examinations also aids in performing interval examinations to look for sequential changes in vocal cord function over time or after intervention.

While this study indicates that laryngeal ultrasound represents a significant advance in the assessment of vocal cord mobility in the infant or pediatric larynx, this is not to suggest that laryngeal ultrasound can replace direct visualization. Clearly, the broader information garnered when performing endoscopy is obvious. However, in the specific assessment of vocal cord mobility in the infant or young child, laryngeal ultrasound can fulfill a critical role.

In summary, most of the available clinical and investigational techniques to evaluate vocal cord mobility in infants and children have significant limitations for practical pediatric clinical use. This study demonstrates that computerized laryngeal ultrasonography is a technique that fulfills most of the requirements of practical clinical tests, namely, the following.

1. Laryngeal ultrasonography is relatively safe, noninvasive, well accepted, and well tolerated.
2. The equipment, while expensive, already exists in most hospital settings, and there is no requirement for additional expense for equipment or technical assistance. The cost of individual studies is reasonable, and the equipment is portable.
3. Data generated by laryngeal ultrasound are easily recorded and stored.
4. Analysis of the data produced by this investigation establishes that laryngeal ultrasound is reliable, accurate, and reproducible, with statistical significance.

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ACKNOWLEDGMENTS — The author recognizes with gratitude the following people, who were enthusiastic and gracious in helping her with this project: Elizabeth Allred, Richard Begin, Arlyn Bonfield, Elizabeth Dubois, Raymond Eckel, Sandra Fernback, MD, Roy McCauley, MD, Jane Share, MD, Rita Teele, MD, and Elizabeth Peabody.

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## THE SEVENTEENTH EUROPEAN INSTRUCTIONAL COURSE ON TINNITUS AND ITS MANAGEMENT

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*Ann Otol Rhinol Laryngol* 106:1997

# PERCEIVED HEARING HANDICAP OF PATIENTS WITH UNILATERAL OR MILD HEARING LOSS

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Using the Hearing Handicap Inventory for Adults (HHIA), we assessed self-perceived hearing handicap in a sample of 63 patients having either unilaterally normal hearing or a mild hearing loss (pure tone average ≤40 dB hearing level). Large intersubject variability in responses to the HHIA confirmed observations that reactions to minimal hearing impairment vary greatly among patients. The individual differences in responses highlight the importance of quantifying the perceived communication and psychosocial handicap, which cannot be determined from the audiogram alone. An item examination of responses to the HHIA revealed a number of emotional and social-situational problems encountered by patients with minimal hearing loss.

KEY WORDS — hearing handicap, hearing impairment, mild hearing loss, unilateral hearing loss.

## INTRODUCTION

Traditionally, adult audiologic rehabilitation has been directed toward remediating the disabilities or handicaps experienced by individuals with hearing impairments of greater than 30 to 40 dB hearing level (HL). Rehabilitative efforts have focused on promoting consistent use of amplification coupled with improvement in communication strategies, including effective listening techniques, environmental management, and speech-reading. More recently, audiologic services have been directed at helping patients with hearing loss of less than 40 dB HL overcome communication and psychosocial handicaps. The interest stems, in part, from the fact that adults with mild high-frequency sensorineural hearing losses are considered candidates for amplification. In fact, patients with minimal hearing loss can adjust to and obtain substantial benefit from hearing aids.[1-3]

While rehabilitative efforts are being directed toward individuals with minimal hearing loss, little information is available describing the impact of this sort of loss on daily function. Garstecki,[4] using the screening version of the Hearing Handicap Inventory for the Elderly,[5] found that 30% of adults passing a pure tone screening experienced some degree of self-perceived hearing handicap.[4] This finding suggests that the perceived problems experienced by his subjects would have been overlooked by the clinician if only audiometric data were used. Using the Self Assessment of Communication, Schow et al[6] reported that hearing handicap measures moved out of the normal range when thresholds exceeded only 25 to 30 dB HL at 1,000 and 2,000 Hz, and with thresholds no poorer than 40 dB HL at 4,000 Hz. In view of these observations, patients with minimal hearing loss and self-perceived handicap have become hearing aid users and should be considered candidates for audiologic intervention.[6,7]

The current investigation was undertaken to examine the impact minimal hearing loss has on an individual's psychosocial function and communication ability in daily life as measured by the Hearing Handicap Inventory for Adults (HHIA). This instrument is a widely used tool because of its brevity, ease of scoring and interpretation, and psychometric adequacy.[8,9] More specifically, the purposes of the present investigation were 1) to quantify self-perceived hearing handicap in a sample of adults with unilateral or mild impairment using the HHIA, and 2) to identify specific emotional and social-situational problems assessed by the HHIA.

## METHODS

*Subjects.* The subjects were 63 adults ranging in age from 18 to 64 years (mean = 48.7; SD = 11.6). The subjects were all outpatients from Henry Ford Hospital undergoing audiologic evaluation. None of the subjects showed evidence of conductive hearing loss as determined by pure tone air and bone conduction audiometry, tympanometry, and otoscopic examina-

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TABLE 1. MEAN BETTER-EAR AND POORER-EAR AIR CONDUCTION THRESHOLDS FOR 63 PATIENTS

| | *Frequency (Hz)* | | | | | |
|---|---|---|---|---|---|---|
| | *250* | *500* | *1,000* | *2,000* | *4,000* | *8,000* |
| Better ear | | | | | | |
| Mean | 16.0 | 18.6 | 19.8 | 28.6 | 49.2 | 52.8 |
| SD | 10.9 | 10.8 | 11.3 | 16.0 | 25.0 | 26.5 |
| Poorer ear | | | | | | |
| Mean | 22.5 | 26.4 | 29.5 | 44.8 | 56.4 | 58.2 |
| SD | 15.9 | 18.6 | 19.7 | 21.1 | 21.1 | 22.9 |

Data are in decibels hearing level.

tion (conducted by an otolaryngologist). None of the subjects reported prior hearing aid use.

In the present investigation, mild hearing loss was defined as a pure tone average (PTA; 500, 1,000, and 2,000 Hz) of ≤40 dB HL in the better ear. Table 1 displays the mean better- and poorer-ear air conduction thresholds. The mean better-ear PTA for the entire sample was 22.8 dB HL (SD = 10). The group was further divided into two subsamples, based on hearing level category determined by the PTA: 1) unilaterally normal (UN; 0 to 25 dB HL), and 2) mild loss bilaterally (26 to 40 dB HL). Forty-three subjects (68%) had UN hearing (mean = 15.8 dB HL; SD = 7.3), and 20 individuals (32%) had mild losses (mean = 32 dB HL; SD = 4.6).

*Materials and Procedures*. The HHIA was employed to quantify self-perceived handicap (Table 2[8]). The HHIA is a 25-item scale composed of a 13-item emotional subscale and a 12-item social-situational subscale. The HHIA was standardized on a sample of adults under 65 years of age and has demonstrated high internal consistency reliability, high test-retest reliability, and a low standard error of measurement.[8,9] A *yes* response to an item is awarded 4 points, a *sometimes* 2 points, and a *no* 0 points. Possible scores for the total scale range from 0, suggesting no perceived handicap, to 100, indicating significant perceived handicap.

Pure tone thresholds were obtained via standard clinical protocol.[10] The HHIA was administered to each subject as part of the routine audiologic evaluation in a face-to-face interview format.

## RESULTS

Table 3[8] displays the mean handicap scores for subjects with UN hearing and mild hearing loss. On independent *t*-tests, no statistically significant differences (p > .05) between groups were observed for the total, emotional, or social-situational scale of the HHIA. The large standard deviations associated with the scores confirm that reactions to minimal hearing loss vary among individuals.

TABLE 2. HEARING HANDICAP INVENTORY FOR ADULTS

| | |
|---|---|
| S-1. | Does a hearing problem cause you to use the phone less often than you would like? |
| E-2. | Does a hearing problem cause you to feel embarrassed when meeting new people? |
| S-3. | Does a hearing problem cause you to avoid groups of people? |
| E-4. | Does a hearing problem make you irritable? |
| E-5. | Does a hearing problem cause you to feel frustrated when talking to members of your family? |
| S-6. | Does a hearing problem cause you difficulty when attending a party? |
| S-7. | Does a hearing problem cause you difficulty hearing/understanding coworkers, clients, or customers? |
| E-8. | Do you feel handicapped by a hearing problem? |
| S-9. | Does a hearing problem cause you difficulty when visiting friends, relatives, or neighbors? |
| E-10. | Does a hearing problem cause you to feel frustrated when talking to coworkers, clients, or customers? |
| S-11. | Does a hearing problem cause you difficulty in the movies or theater? |
| E-12. | Does a hearing problem cause you to be nervous? |
| S-13. | Does a hearing problem cause you to visit friends, relatives, or neighbors less often than you would like? |
| E-14. | Does a hearing problem cause you to have arguments with family members? |
| S-15. | Does a hearing problem cause you difficulty when listening to TV or radio? |
| S-16. | Does a hearing problem cause you to go shopping less often than you would like? |
| E-17. | Does any problem or difficulty with your hearing upset you at all? |
| E-18. | Does a hearing problem cause you to want to be by yourself? |
| S-19. | Does a hearing problem cause you to talk to family members less often than you would like? |
| E-20. | Do you feel that any difficulty with your hearing limits or hampers your personal or social life? |
| S-21. | Does a hearing problem cause you difficulty when in a restaurant with relatives or friends? |
| E-22. | Does a hearing problem cause you to feel depressed? |
| S-23. | Does a hearing problem cause you to listen to TV or radio less often than you would like? |
| E-24. | Does a hearing problem cause you to feel uncomfortable when talking to friends? |
| E-25. | Does a hearing problem cause you to feel left out when you are with a group of people? |

Modified from Newman et al.[8]
S — social-situational subscale, E — emotional subscale.

In order to examine the degree of handicap experienced by the subjects more closely, the hearing handicap categories used for the HHIA were employed: 0 to 16 points, no handicap; 18 to 42, mild to moderate handicap; and over 42, significant handicap. Figure 1[8] graphically displays the proportion of subjects with UN hearing and mild losses falling

TABLE 3. SCORES ON HHIA FOR PATIENTS WITH UNILATERALLY NORMAL HEARING AND MILD HEARING LOSSES

| | HHIA | | |
|---|---|---|---|
| *Hearing Level Category* | *Total* | *Emotional* | *Social-Situational* |
| Unilaterally normal (0-25 dB HL; n = 43) | | | |
| Mean | 33.5 | 16.9 | 16.6 |
| SD | 21.8 | 12.2 | 10.6 |
| Mild loss (26-40 dB HL; n = 20) | | | |
| Mean | 41.9 | 21.5 | 20.4 |
| SD | 33.5 | 14.5 | 12.3 |

For between-group comparisons, $p > .05$.

HHIA — Hearing Handicap Inventory for Adults.[8]

within each of the handicap categories. It is noteworthy that when the data are dichotomized into "no handicap" (0 to 16 score) versus "handicap" (over 16 score) categories, three quarters of the subjects with both UN hearing and mild losses reported some degree of communication and psychosocial problems.

An item examination of the HHIA was performed in order to help isolate specific emotional and social-situational problems encountered by each subgroup. Responses to individual items were dichotomized into "no handicap" (a *no* response to an item) and "handicap" (a *sometimes* or *yes* response to an item) categories. Subsequently, the proportion of individuals responding to a particular item as handicapping was calculated.

Figure 2[8] displays the endorsement rates for individual items on the HHIA. Figure 2A shows the percentage of subjects with UN hearing and mild losses reporting a handicap for individual items on the emotional subscale of the HHIA. For subjects with UN hearing, items relating to feeling frustrated, upset, and left out had the three highest endorsement rates. For subjects with mild loss, items associated with irritability, feeling upset, and feeling left out of groups were perceived as the three greatest emotional consequences of hearing loss. Figure 2B displays the percentage of subjects with UN hearing and mild hearing loss reporting handicap for individual items on the social-situational subscale. For both groups, the highest endorsement rates were associated with attending a party, listening to television or radio, and being at a restaurant.

## DISCUSSION

The current investigation was prompted by clinical observation and a few studies[4,6,11,12] suggesting that despite the audiometric appearance of a minimal hearing loss, individuals are handicapped by hearing levels of less than 40 dB HL. The large intersubject variability observed for the total, emotional, and social-situational scales of the HHIA in the present study lends further support to the clinical observations that individuals react differently to similar audiometric configurations. For example, 1 SD (33.5) from the mean (41.9) for the total score for the mild loss group represents scores ranging from 8.4 to 75.4. These scores indicate that some subjects reported "no handicap" while others reported "significant handicap." It has been suggested that variability in self-report handicap scores may be due to a number of extra-audiologic variables such as personality, general health, and life-style.[13,14]

Our findings emphasize the need to consider the initiation of rehabilitative intervention with minimally impaired adults when the patient first begins to notice difficulty. It has been suggested that early intervention with adults before retirement age may promote greater acceptance of hearing loss and amplification later in life.[15]

Because of the imperfect relationship between

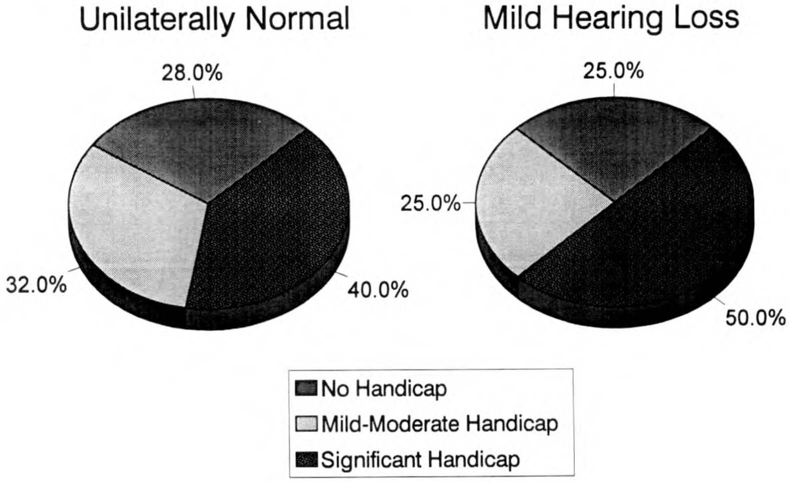

Fig 1. Proportion of patients with unilaterally normal hearing (n = 43) and mild losses (n = 20) falling within each of handicap categories on Hearing Handicap Inventory for Adults (HHIA).[8]

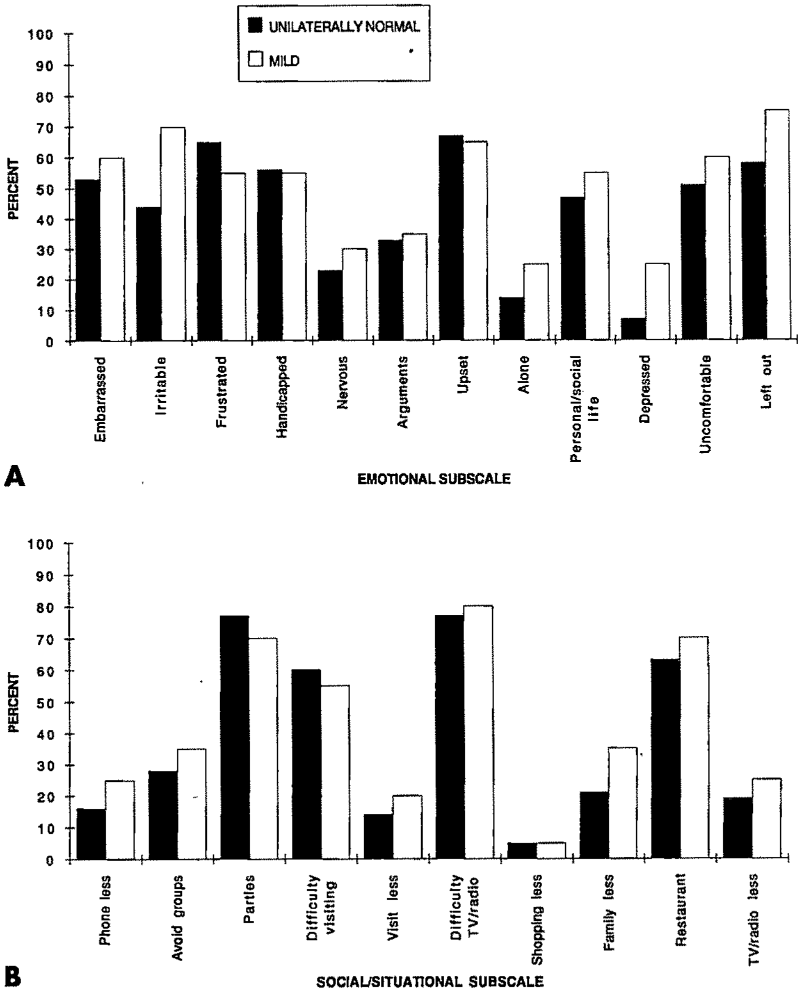

Fig 2. Percentage of patients with unilaterally normal hearing and mild losses reporting handicap for individual items on A) emotional subscale and B) social-situational subscale of HHIA.[8]

hearing impairment (eg, audiometry) and handicap (self-report measures), it has been suggested by the American Speech-Language-Hearing Association[16] that in screening adults, a test of hearing handicap may be as important as or more important than a measure of impairment. By screening for both hearing impairment and hearing handicap, it would be possible to identify potential candidates for rehabilitative services who might have been missed by pure tone screening alone. In this connection, Bennett[1] fit 98 patients with amplification who had hearing levels of <20 dB HL at 500 and 1,000 Hz, and ≤35 dB HL at 2,000 Hz (patients who traditionally have not been considered candidates for amplification or rehabilitative intervention). After the 30-day trial period, 92% of the patients elected to purchase the hearing aids. At the end of 6 months, 85% of the patients considered the hearing aids a worthwhile investment. Therefore, if candidacy for amplification had been based solely on pure tone thresholds, 76 patients would not have been fitted with amplification and subsequently would not have benefited from wearing hearing aids.[1]

Newman et al[2] also illustrated the imperfect relationship between handicap and impairment by administering a self-report handicap measure in a prefitting and postfitting paradigm to a sample of individuals with hearing losses ranging from mild to severe. Subjects with hearing loss of greater than 40 dB HL demonstrated mean prefitting and postfitting handicap scores similar to those of subjects with hearing losses of less than 40 dB HL. The magnitude of hearing aid benefit, thus, was not affected by the degree of impairment. These results, coupled with the results of Bennett[1] and others,[17] suggest that candidacy for hearing aids involves both perceived handicap and audiometric data. Accordingly, the degree of communication difficulty in patients with audiometrically mild hearing losses cannot be predicted from the audiogram.

An item examination of the HHIA was conducted to determine to what extent and in what situations patients with UN hearing and mild hearing loss experienced difficulties. More than 50% of the present sample reported problems listening in the presence of background noise (eg, parties, restaurant, visiting) and when using a television or radio. These observations emphasize the need to provide appropriate counseling to the patient regarding communication strategies and the consideration of assistive listening devices (ALDs) even when hearing aids may not be recommended. For example, patients may benefit from remote microphone technology (eg, FM system) to help overcome problems associated with a poor signal-to-noise ratio in listening situations. It may well be that adults with minimal impairment can begin the rehabilitative process with ALDs in selected situations and "graduate" to personal hearing aids once they are exposed to the benefits derived from amplification.

In conclusion, the individual variability in response to minimal impairment highlights the importance of assessing the psychosocial consequences of hearing loss in order to facilitate appropriate intervention. Our findings indicate that UN or mildly impaired adults should be considered candidates for audiologic rehabilitation, including at least patient-family counseling regarding communication strategies and the option to evaluate the potential benefits from amplification.

ACKNOWLEDGMENT — We thank Dr Barbara Weinstein for her helpful comments and suggestions on an earlier version of the paper.

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*Ann Otol Rhinol Laryngol* 106:1997

# INCREASE IN EXPRESSION OF IL-4 AND IL-5 MRNA IN THE NASAL MUCOSA OF PATIENTS WITH PERENNIAL ALLERGIC RHINITIS DURING NATURAL ALLERGEN EXPOSURE

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To understand the role of cytokines in allergic nasal disease in humans, we designed this study to investigate the expression of cytokine mRNA in the nasal mucosa of 10 control subjects and 10 patients with perennial allergic rhinitis using a reverse transcription–polymerase chain reaction (RT-PCR) for IL-4, IL-5 (Th2 cytokines), and IFN-γ (Th1 cytokine). Both IL-4 and IL-5 mRNAs were expressed in a significantly larger number of patients with allergic rhinitis than in control subjects. When the levels of expression of cytokines and β-actin mRNA were quantitated, the mean density ratios (cytokine/β-actin) for IL-4 and IL-5 were higher in patients with allergic rhinitis than in control subjects. In contrast, there were no differences in the number of subjects who showed expression of IFN-γ mRNA, or in mean density ratios for IFN-γ between the two groups. This study suggests that both IL-4 and IL-5 mRNAs are expressed almost universally in the nasal mucosa of patients with perennial allergic rhinitis during natural allergen exposure and also that Th2-type cytokines play a crucial role in the development of allergic nasal diseases in vivo.

KEY WORDS — cytokines, nasal mucosa, natural allergen exposure, perennial allergy.

## INTRODUCTION

Allergic rhinitis is an inflammation of the nasal mucosa that is characterized by infiltration of inflammatory cells and the release of inflammatory mediators. The pathophysiologic mechanism of nasal allergy is not well understood. There has recently been much interest in the role of cytokines in allergic inflammatory reactions. Numerous in vitro studies have suggested that many cytokines play an important role in the pathogenesis of allergic inflammatory diseases.[1-3] Interleukin (IL)–4 is required for the production of IgE by inducing an isotype switch in B cells, and this function of IL-4 is inhibited by IFN-γ.[3,4] Interleukin-5 stimulates the growth and differentiation of eosinophils and increases the survival of eosinophils as well as the production of immunoglobulin A levels in murine spleen B cells.[5,6] The development and activation of mast cells are related to IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF).[7,8]

However, only limited information is available on the pattern of cytokine expression in vivo[9,10] and on the difference in cytokine expression in nasal mucosa between patients with allergic rhinitis and subjects with no nasal allergy. Furthermore, there are few reports on cytokine expression in patients with perennial allergic rhinitis, especially during natural allergen exposure. In the present study, in order to help us understand the role of cytokines in the development of nasal allergy, expression of cytokine messenger RNAs (mRNAs) was investigated in the nasal mucosa of 10 control subjects and 10 patients with perennial allergic rhinitis by means of a reverse transcription–polymerase chain reaction (RT-PCR) for IL-4, IL-5 (Th2 cytokines), and IFN-γ (Th1 cytokine).

## MATERIALS AND METHODS

*Subjects*. Ten adult patients (6 men, 4 women, 19 to 60 years of age) with perennial allergic rhinitis were selected on the basis of a positive clinical

TABLE 1. PRIMER SEQUENCES

| | *Upstream (5' Primer)* | *Downstream (3' Primer)* |
|---|---|---|
| IL-4 | 5'ATGGGTCTCACCTCCCAACTGCT3' | 5'CGAACACTTTGAATATTTCTCTCTCAT3' |
| IL-5 | 5'GCTTCTGCATTTGAGTTTGCTAGCT3' | 5'TGGCCGTCAATGTATTTCTTTATTAAG3' |
| IFN-γ | 5'GCATCGTTTTGGGTTCTCTTGGCTGTTACTGC3' | 5'CTCCTTTTTCGCTTCCCTGTTTTAGCTGCTGG3' |
| β-actin | 5'ATCTGGCACCACACCTTCTACAATGAGCTGCG3' | 5'CGTCATACTCCTGCTTGCTGATCCACATCTGC3' |

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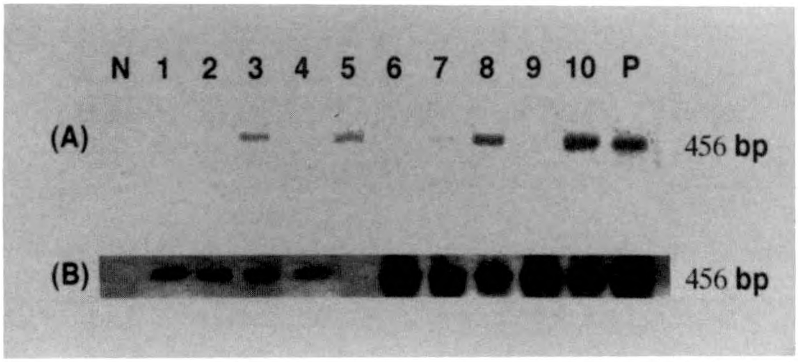


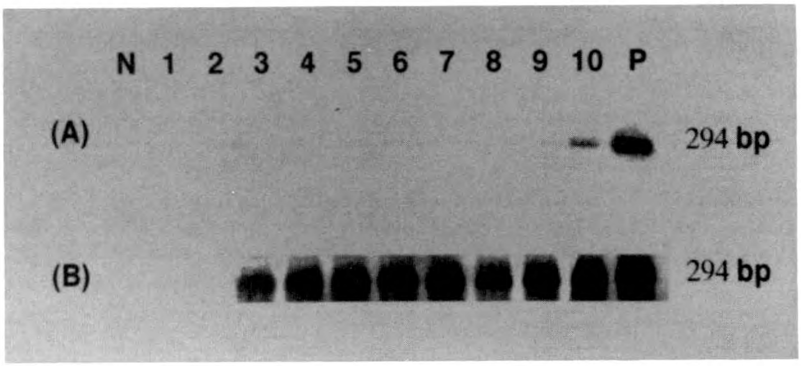
Fig 1. Southern blot analysis for IL-4 RT-PCR products. cDNA was synthesized from total RNA and amplified with primers specific for IL-4. RT-PCR products were blotted and hybridized with IL-4 probe. Nine of 10 subjects showed expression of IL-4 mRNA in allergy group (B), while 4 of 10 subjects showed expression of IL-4 mRNA in control group (A). Human IL-4 cDNA was used as positive control (P). As negative control, sterile distilled water was used (N).

history, a positive skin prick test, and/or a positive radioallergosorbent test (RAST) to house dust mite (*Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*). The patients were excluded if they had previously received immunotherapy, or were taking regular topical or oral medication. The patients were not studied within 3 weeks after recovering from an upper respiratory tract infection. Ten healthy volunteers (7 men, 3 women, 22 to 54 years of age) were selected as controls after informed consent was obtained. None of the subjects had any sign of acute infection, nor did they receive antibiotics. To assess the cytokine gene expression in allergic nasal mucosa during natural exposure to allergens, we obtained nasal mucosal specimens from the inferior turbinate without artificial nasal allergen challenge.

*Reverse Transcription–Polymerase Chain Reaction.* The biopsy specimens were frozen immediately in liquid nitrogen and were stored at –70°C. Total RNA was extracted by the guanidinium-phenol-chloroform extraction method.[11] Each RNA solution prepared as above was reverse-transcribed into complementary DNA (cDNA) with the Gene-Amp RNA-PCR kit (Perkin-Elmer Cetus Corporation, Branchburg, NJ). The PCR was carried out with cDNA in a DNA thermal cycler (Perkin-Elmer Cetus Corporation). Twenty microliters of cDNA were amplified with AmpliTaq DNA polymerase (Perkin-Elmer Cetus Corporation) according to the manufacturer's PCR protocol. Hot-start PCR was carried out for 35 cycles. The conditions for PCR were as follows: 1 minute at 95°C for the denaturation step, 1 minute at 55°C for the annealing step, and 1 minute at 72°C for the ex-tension step. Primer sequences of each cytokine and β-actin used in this study are listed in Table 1. The predicted sizes of amplified fragments of IL-4, IL-5, IFN-γ, and β-actin were 456, 294, 427, and 838 base pairs, respectively. The control cDNA of each cytokine and β-actin (Clontech Laboratories, Palo Alto, Calif) was used as a positive control in the PCR procedure. As a negative control, sterile distilled water was added to the reaction mixture instead of cDNA at the RT step. The specificity of PCR product for each cytokine was detected by Southern blot analysis.

*Southern Blot Analysis of Amplified cDNA.* Amplified cDNA was separated by electrophoresis through a 1.5% agarose gel and was transferred to nylon membrane by means of the capillary diffusion method. Nylon membrane containing target DNA was prehybridized at 42°C for 3 hours and hybridized at 55°C overnight in 50% formamide with 5× standard saline citrate (SSC), 2% blocking solution (Boehringer Mannheim, Mannheim, Germany), 0.1% N-lauroyl sarcosine, and 0.02% sodium dodecyl sulfate (SDS). Target DNA was detected with a digoxigenin-labeled probe that was synthesized by PCR with control cDNA of each cytokine as a template. The blot was washed in 2× SSC and 0.1% SDS at room temperature for 5 minutes to repeat two times, and then, 0.1× SSC and 0.1% SDS at 68°C for 15 minutes two times. A digoxigenin DNA labeling and detection kit (Boeh-

Fig 2. Southern blot analysis for IL-5 RT-PCR products. cDNA was synthesized from total RNA and amplified with primers specific for IL-5. RT-PCR products were blotted and hybridized with IL-5 probe. Eight of 10 subjects showed expression of IL-5 mRNA in allergy group (B), while 1 of 10 subjects showed expression of IL-5 mRNA in control group (A). Human IL-5 cDNA was used as positive control (P). As negative control, sterile distilled water was used (N).



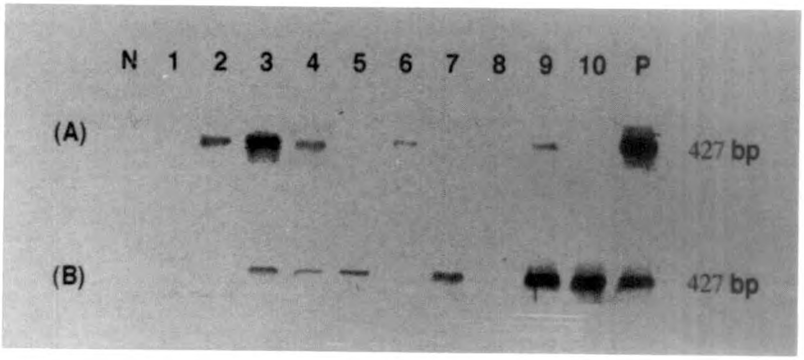


Fig 3. Southern blot analysis for IFN-γ RT-PCR products. cDNA was synthesized from total RNA and amplified with primers specific for IFN-γ. RT-PCR products were blotted and hybridized with IFN-γ probe. Six of 10 allergic subjects (B) and 5 of 10 control subjects (A) showed expression of IFN-γ mRNA, respectively. Human IFN-γ cDNA was used as positive control (P). As negative control, sterile distilled water was used (N).

ringer Mannheim) for nucleic acids was used to detect the bound probe by colorimetry. The band intensity of amplification products for each cytokine and β-actin obtained from RNA of individual subjects was quantitated with a densitometer. The density ratio was calculated by dividing the intensity of the band of each cytokine by that of β-actin in the same subject.[12]

## RESULTS

The amplification products of IL-4, IL-5, IFN-γ, and β-actin were identical to the expected size for the mRNA template of 456, 294, 427, and 838 base pairs, respectively. When sterile distilled water was used as a negative control, no band was observed. The expression of IL-4 mRNA was observed in 9 of 10 allergic patients (90% positive) and in 4 of 10 control subjects (40% positive; Fig 1). Among the 10 patients with nasal allergy examined in this study, the band for IL-5 mRNA was observed in 8 patients (80% positive), whereas IL-5 mRNA was expressed in only 1 of 10 control subjects (10% positive; Fig 2). In contrast, there was no difference in expression of IFN-γ mRNA between allergic patients and control subjects. Six of 10 allergic patients (60%) and 5 of 10 control subjects (50%) expressed IFN-γ mRNA, respectively (Fig 3). The number of subjects who showed expression of IL-4 and IL-5 mRNA was greater in allergic patients than in control subjects (Table 2). The bands of β-actin were observed in all of the samples (Fig 4).

As listed in Table 3, the means ± SD of the IL-4/β-actin and IL-5/β-actin density ratios were 0.97 ± 0.23 and 0.69 ± 0.46 for allergic patients and 0.46 ± 0.25 and 0.27 ± 0.00 for control subjects, respectively. (The SD was zero because only 1 control subject showed expression of IL-5 mRNA.) The mean density ratios for IL-4 and IL-5 in allergic patients were higher than those in control subjects (Table 3). However, there was no difference in the IFN-γ/β-actin density ratio between the two groups. The mean ± SD of the IFN-γ/β-actin density ratios was 0.42 ± 0.10 for allergic patients and 0.38 ± 0.09 for control subjects. These results were consistent with the results obtained from the number of subjects who showed expression of cytokine mRNAs.

TABLE 2. NUMBER OF SUBJECTS WITH POSITIVE BAND ON SOUTHERN BLOT ANALYSIS OF AMPLIFIED cDNA

| | *Allergic Patients (n = 10)* | *Control Subjects (n = 10)* |
|---|---|---|
| IL-4 | 9 | 4 |
| IL-5 | 8 | 1 |
| IFN-γ | 6 | 5 |

## DISCUSSION

Of the Th2-type cytokines, IL-4 and IL-5, investigated in this study, are thought to play the most important roles in the development of allergic diseases. The results of this study show that both IL-4 and IL-5 mRNAs are almost universally expressed in the nasal mucosa of patients with perennial allergic rhinitis without nasal provocation. In comparing the expression of IL-4 and IL-5 mRNAs, it can be suggested that IL-5 mRNA is more specifically expressed in the nasal mucosa of patients with allergic rhinitis, because only 1 of 10 control subjects showed expression of IL-5 mRNA, whereas 4 of 10 control subjects showed expression of IL-4 mRNA. Terada et al[9] recently demonstrated expression of IL-5 mRNA in 2 out of 4 subjects before allergen challenge and in 3 subjects after allergen challenge in the nasal mucosa of patients with perennial allergy to house dust mites. In contrast, our study demonstrated that 8 of 10 patients with perennial allergic rhinitis showed expression of IL-5 mRNA in the nasal mucosa, even though nasal provocation with specific allergen was not performed. This difference in the expression rate of IL-5 mRNA may be due to the small study population in the study of Terada et al or a different density of house dust mites in the indoor environment in Japan versus Korea.

In Korea, the house dust mite is by far the most common offending allergen in allergic rhinitis.[13] Thus, patients sensitive to house dust mites were selected as study subjects in this study. Nasal provocation with allergen was not performed before taking

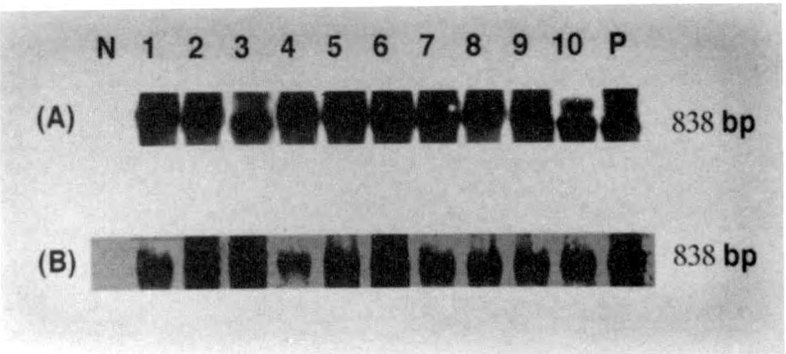


Fig 4. Southern blot analysis for β-actin RT-PCR products. cDNA was synthesized from total RNA and amplified with primers specific for β-actin. RT-PCR products were blotted and hybridized with β-actin probe. All subjects in both groups (A: control, B: allergy) showed positive expression of β-actin mRNA. Human β-actin cDNA was used as positive control (P). As negative control, sterile distilled water was used (N).

biopsy specimens for the following reasons. First, the authors assumed that cytokines involved in the development of allergic rhinitis would be expressed without nasal provocation, because perennial allergens like house dust mites would stimulate the nasal mucosa more or less constantly throughout the year. Second, the amount of allergen introduced during the nasal provocation procedure is much larger than that introduced in a state of natural allergen exposure. Thus, the nasal allergen challenge may influence the results of the study and lead to overestimation or underestimation of the role of certain kinds of cytokines in the nasal mucosa in vivo. In fact, the profile of inflammatory cell infiltration was different between the studies done during natural allergen exposure[14] and after nasal provocation with allergen.[15]

Another study[16] suggested that IL-4 producing nasal memory T cells existed on site in the nasal mucosa of allergic subjects after provocation with allergen. However, it was difficult to conclude whether the nasal T cells were recruited from the blood or reside in the nasal mucosa. Soluble antigens and some helminth components promote the differentiation of Th cells into the Th2 phenotype.[17] Thus, Th2 cells also appear to be the critical factor for the development of an allergic response in nasal mucosa in vivo. It is also probable that non-T cells play an important role in initiating the allergic inflammatory reaction through their ability to secrete mediators rapidly, because the cytokine production by T cells after stimulation takes several hours and because the initial induction of Th2 cells requires IL-4. However, we do not have any evidence about which type of cell is at the center of allergic inflammatory responses. The presence of mRNA for a protein does not always indicate translation into product, so the demonstration of cytokine product within cells is an important observation, and we do not have any evidence about the source of cells producing these cytokines in nasal mucosa, because either T cells or non-T cells (mast cells or basophils) can produce IL-4 and IL-5. However, this study would suggest that there might be T cells or non-T cells that can produce these cytokines in nasal mucosa of patients with perennial allergic rhinitis even if allergen provocation is not performed. The results of several studies and this observation suggest that these cytokines contribute to the development or maintenance of nasal allergic inflammation in patients with perennial allergic rhinitis, and may enable a new therapeutic approach in the treatment of allergic rhinitis to be developed by modulating the expression of Th2 cytokines.

In conclusion, the aim of this study was to elucidate the pattern of cytokine mRNA expression in allergic nasal mucosa under the conditions of natural allergen exposure. In this study, although the expression of cytokine mRNAs could possibly be potentiated by nasal provocation with allergen, we have demonstrated the increased expression of Th2-type cytokines, IL-4 mRNA and IL-5 mRNA, in patients with perennial allergic rhinitis during natural allergen exposure. However, there was no difference in expression of IFN-γ mRNA, a Th1-type cytokine, between nasal allergic patients and control subjects. The results of this study suggest that Th2-type cytokines may play a more crucial role in the pathophysiology of allergic rhinitis in vivo.

TABLE 3. DENSITY RATIO OF POSITIVE BANDS IN EACH GROUP

| | *Allergic Patients* | *Control Subjects* |
|---|---|---|
| IL-4 | 0.97 ± 0.23 | 0.46 ± 0.25 |
| IL-5 | 0.69 ± 0.46 | 0.27 ± 0.00 |
| IFN-γ | 0.42 ± 0.10 | 0.38 ± 0.09 |

Density ratio is expressed as mean ± SD; density ratio = A/B, where A = density of band of each cytokine on Southern blot measured by densitometry, and B = density of β-actin of same subjects.

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## INTERAMERICAN ASSOCIATION OF PEDIATRIC OTORHINOLARYNGOLOGY

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*Ann Otol Rhinol Laryngol* 106:1997

# DISTORTION PRODUCT EMISSIONS IN NORMAL-HEARING AND LOW-FREQUENCY HEARING LOSS CARRIERS OF GENES FOR WAARDENBURG'S SYNDROME

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Eight patients with Waardenburg's syndrome (WS) with normal hearing and 3 additional patients exhibiting a low-frequency hearing loss were tested for the level of the acoustic distortion product 2f1–f2 by means of the Otodynamics Distortion Product Analyser (ILO92). Wide notches in distortion product otoacoustic emissions (DPOAEs) between 1,000 and 3,000 Hz were found in 7 (12 ears, 87.5%) examined patients with normal audiograms, which was a significantly higher rate than that found in the control group (10%). The 3 patients with low-frequency hearing loss gave a consistent pattern in audiometric configuration shown by both pure tone audiograms and DPOAEs. It is concluded from these initial results that DPOAEs may be a useful approach to identifying subclinical pathologic aberrations in the inner ear in WS patients, and may be a predictor of low-frequency sensorineural hearing loss.

KEY WORDS — distortion product otoacoustic emissions, low-frequency hearing loss, normal hearing, Waardenburg's syndrome.

## INTRODUCTION

Waardenburg's syndrome (WS), characterized by congenital sensorineural hearing loss and pigmentary disturbances of the eyes, hair, and skin, is one of the most common syndromal conditions causing hearing impairment.[1] Clinically, WS is divided into two distinct types based on the presence (type I) or absence (type II) of dystopia canthorum (lateral displacement of the inner canthi).[2,3] Genetic linkage studies have localized the gene for WS type I to the distal region of chromosome 2q[4] and one gene for type II to chromosome 3p.[5] Further studies have demonstrated that mutations in the PAX3 gene can cause type I and that mutations in the MITF gene result in type II.[6,7]

Congenital sensorineural hearing loss is the most significant clinical feature of the syndrome, as it has a functional implication for the affected individual. Penetrance of hearing loss was found in 36% to 69% of type I individuals and in 57% to 87% of type II.[3,8,9] Although the audiologic features of WS are highly variable, with bilateral and unilateral involvement and slight to profound hearing impairment, many individuals with WS escape the auditory effects of the mutations due to the difference in expression of WS genes.[8,10] Some normally hearing individuals with WS give birth to hearing-impaired children. The reason for the interesting difference is not clear. However, it is possible that conventional audiometric methods (such as pure tone audiometry) are not sufficiently sensitive to detect small audiometric signs of a cochlear defect, and that a more sensitive approach might indicate some evidence of a gene effect. This would be of particular value in type II, in which the major diagnostic sign of WS type I, wide spacing between the eyes, is absent, and diagnosis is correspondingly more difficult.[10]

Low-frequency hearing loss is commonly seen in patients with WS, and is found in 14.4% of WS audiograms.[9] This specific audiogram profile is also seen in nonsyndromic hearing loss and other known causes of hearing loss.[11,12] Although the auditory brain stem response (ABR) is the most reliable and accurate hearing test for infants available at present, a normal ABR would not necessarily exclude a low-frequency hearing loss, and such infants have to be followed up until they can be tested with behavioral methods.

Distortion product otoacoustic emissions (DPOAEs) are defined as acoustic energy in the ear canal arising from the nonlinear interaction of two simultaneously applied pure tones within the cochlea.[13] It is proposed that measurement of DPOAEs would provide valuable information concerning cochlear status and might reflect a genetically determined structural factor.[14,15] It is possible that subclinical changes that are not detectable with conventional audiometry might be revealed by particular characteristics of an individual's DPOAEs. One method of recording DPOAEs is a "distortion product (DP)–gram," in which emission amplitudes in response to constant-level primary tones (eg, $L_1 = L_2 =$ 70 dB sound pressure level) are measured as a function of regular increments in stimulus frequency. In

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producing DP-grams, these emissions are usually evoked by primaries with geometric mean frequencies between 0.5 and 8 kHz from 1 to 16 points per octave, depending on the desired frequency resolution. The DP-gram furnishes detailed information about the frequency pattern of emission activity.

There is now considerable evidence that DPOAEs are generated from very specific cochlear sites.[16,17] This cochlear place specification of DPOAEs may lead to a frequency-specific relationship with cochlear function.[18,19] The implication of the frequency-specific properties of DPOAEs is that each part of the cochlea can be characterized by looking at the amplitude on the DP-gram. Because they have greater inherent frequency specificity than transient evoked otoacoustic emissions, DPOAEs have attracted special interest for their potential use as objective predictors of the pure tone hearing threshold.

Several studies in the past investigated obligate and possible carriers of various forms of genetic hearing loss by using conventional audiometry (pure tone audiometry, Bekesy audiometry, and stapedial reflex). Some studies have suggested that these audiometry techniques may help to indicate heterozygosity; that is, audiometric "dips" may be present in individuals who are functionally normal-hearing but carry genes that cause a hearing loss. Anderson and Wedenberg[20] used Bekesy audiometry to obtain audiograms from 30 subjectively well-hearing parents of children with autosomal recessive hearing loss. They reported that there was a greater prevalence of audiometric dips wider than one octave in obligate carriers than in controls. This finding suggests that the carriers of the recessive genes for hearing loss may have functional aberrations in their hearing organ and that an audiometric "dip" may be present in individuals who are functionally normally hearing but carry genes that cause a hearing loss. Stolbova and Valvoda[21] examined 13 normal-hearing carriers of the genes for autosomal dominant nonsyndromic hearing loss, and showed changes in their hearing function. A pathologically elevated threshold of stapedial reflexes and positive recruitment were found among 13 carriers of the gene with subjectively good hearing and normal audiograms. Their work disagreed with the investigation of Taylor et al.[22] A recent study involving the examination of a few families using a new sweep frequency technique, Audioscan, concluded that all obligate carriers of a gene causing Usher's syndrome type II have audiometric notches in the frequency range 500 to 3,000 Hz and so do the expected proportion of possible carriers.[23] It is claimed that the Audioscan technique may offer a sensitive technique for the detection of carriers in families with Usher's syndrome type II.

Some authors have measured the DPOAEs of individuals who have been exposed to some factors causing hearing impairment but who had normal pure tone audiograms. Gaskill and Brown[24] found that some such individuals had abnormal DPOAEs. This suggested that hearing impairment in these patients not detected by conventional audiometry was detectable in DPOAEs. Lonsbury-Martin et al[15] used DPOAEs to test a subject who had experienced a gun blast in the right ear, and compared the DPOAE audiogram with his pure tone audiogram. They also found that although the pure tone threshold was in the normal range, the DPOAEs showed a high-frequency cochlear defect. This suggested that DPOAEs could detect an underlying condition in which the hair cells were gradually deteriorating before this deterioration was revealed as a threshold elevation.

In the present study we used ILO92 to measure the DPOAEs from 8 WS patients (16 ears) with normal hearing and 3 WS patients (5 ears) with low-frequency hearing loss. The usefulness of a DP-gram in identifying those normally hearing individuals affected by WS genes and in predicting low-frequency hearing loss is explored.

## METHODS

*Subjects*. The control group comprised 16 male and 14 female subjects (60 ears) ranging in age from 5 to 60 years (mean 31 years). They were obtained from among staff of the University of Manchester and from among friends or non-blood relatives of patients attending the study. None of these subjects considered that they had any hearing problems.

Patients were taken from a group of WS patients in our research program.[10] All individuals met the diagnostic criteria for WS type I and type II suggested by Farrer et al[25] and Liu et al.[10] Patient group 1 comprised 8 patients (3 type I from 2 families and 5 type II from 2 families). Their mean age was 34 years (range 4 to 60 years). None of these patients complained of hearing loss. Patient group 2 included 3 WS patients (2 type I and 1 type II) with unilateral or bilateral low-frequency hearing loss (2 males and 1 female). Their ages ranged from 18 years to 45 years.

*Procedure*. The clinical protocol included a pedigree, a questionnaire, and physical and audiologic examinations.[10] All subjects were otoscopically examined, and pure tone audiometry and otoadmittance measurements were conducted on all subjects. Air conduction thresholds were measured at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. A GSI 28A Auto Tympanometer was used for tympanometry and acoustic reflex measurements. The compliance of the tympanic membrane, the value of the middle

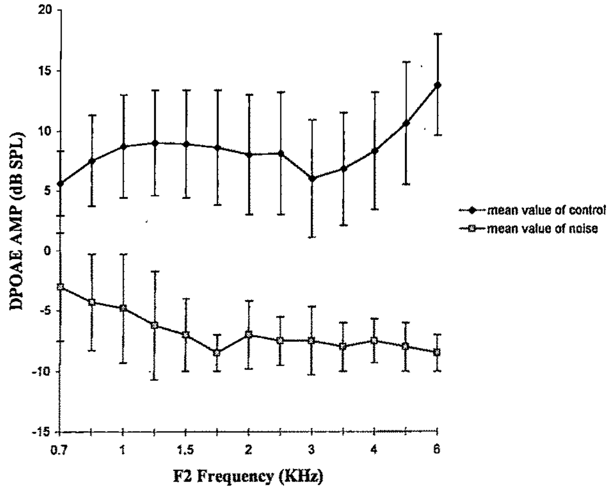

Fig 1. Mean distortion product otoacoustic emission (DPOAE) amplitude (AMP) values recorded from 30 subjects in control group. L1 = L2 = 70 dB sound pressure level (SPL). Error bars — ±1 SD.

ear pressure, and acoustic reflex thresholds were recorded on a tympanogram allowing an evaluation of middle ear function.

Testing of DPOAEs was performed with the ILO92 Distortion Product Analyser (Otodynamics UK 1992). The subject was seated and asked to remain still in a sound-treated room. A frequency resolution of 4 points per octave was chosen. Distortion product measurements were made over a specific number of frequencies: 700 Hz, 1 kHz, 1.5 kHz, 2 kHz, 3 kHz, 4 kHz, 5 kHz, and 6 kHz. These stimulus tones are varied across a wide range of frequencies and the 2f1–f2 distortion product is plotted as a function of the f2 stimulus. A probe containing three transducers — two loudspeakers and one microphone — was inserted into a foam tip and placed in the patient's external ear canal. The intensity of each stimulus was set at 70 dB sound pressure level.

With the ILO92 analyzer, the narrow, sharp notch is defined as a sharp decrease in the amplitude of a DPOAE, followed by a sharp recovery (eg, one point touches the noise floor). The wide notch is defined as a sharp decrease in the amplitude, extending over a wide frequency range (eg, 500 Hz), followed by a sharp recovery (eg, two points or more touch the noise floor).

## RESULTS

*Controls.* All subjects had the following findings:

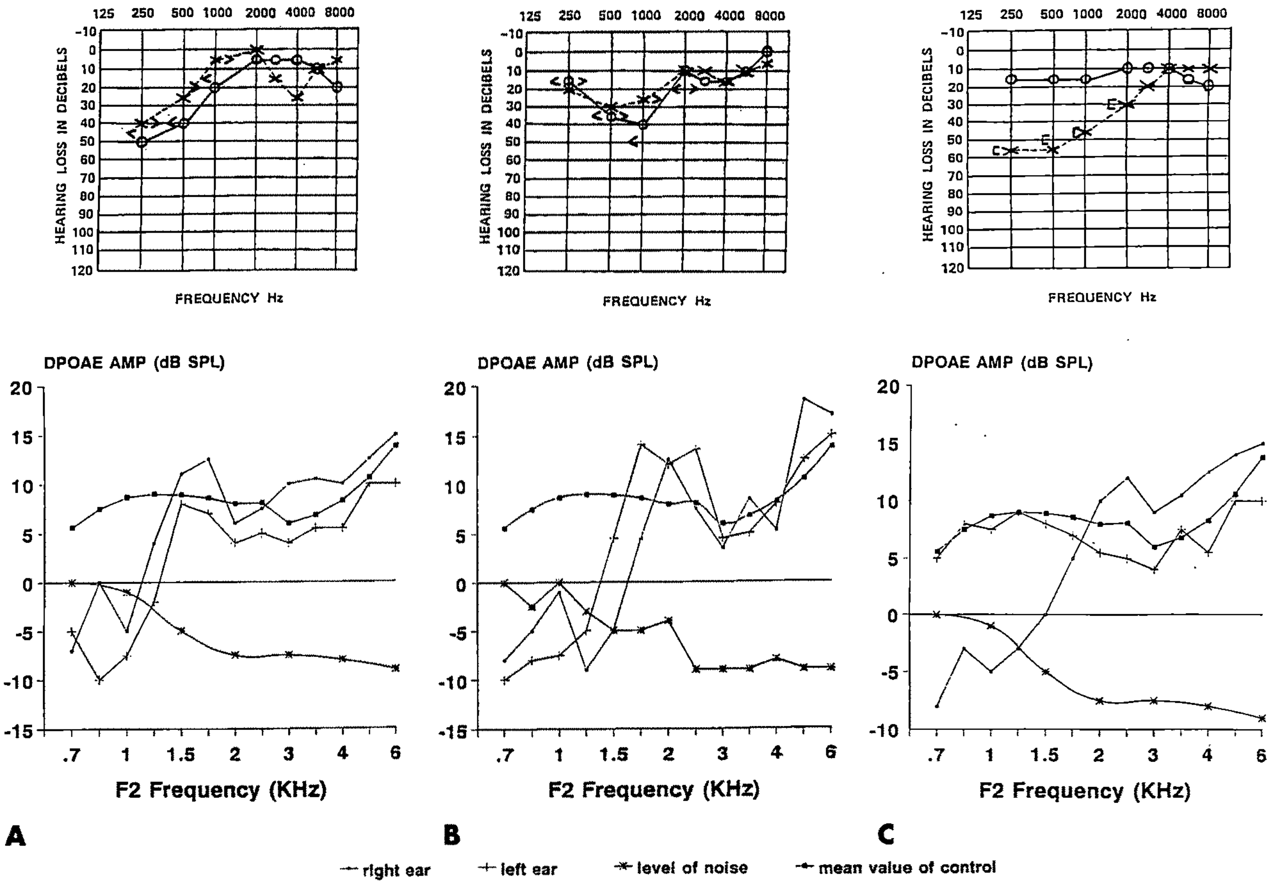

Fig 2. Distortion product (DP)–grams and audiograms obtained from 3 patients with low-frequency loss. A) Type I Waardenburg's syndrome (WS) patient with bilateral hearing loss. B) Type II WS patient with bilateral hearing loss. C) Type I WS patient with unilateral hearing loss. L1 = L2 = 70 dB SPL.

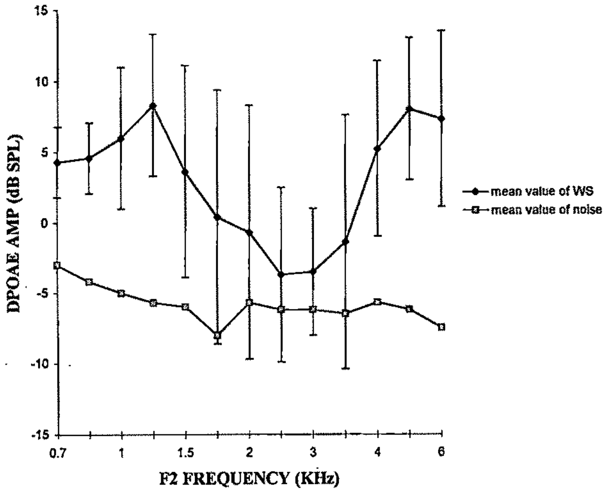

Fig 3. Mean DPOAE amplitude values measured from 7 patients (12 ears) with wide notches. L1 = L2 = 70 dB SPL. Error bars — ±1 SD.

1) air conduction thresholds of no poorer than 15 dB hearing level at octave frequencies between 250 Hz and 8 kHz and normal middle ear function, 2) no significant history of noise exposure or ototoxic drugs, and 3) no family history of hearing loss or pigmentary disturbances.

Four (8 ears) of 30 subjects demonstrated the occurrence of a deep notch in the amplitude in the frequency range 1,000 to 3,000 Hz. Of them, 1 subject had sharp notches, and 3 subjects had wide notches as judged by the ILO92. Figure 1 shows the mean DPOAE amplitude values obtained at each frequency from 30 subjects (60 ears).

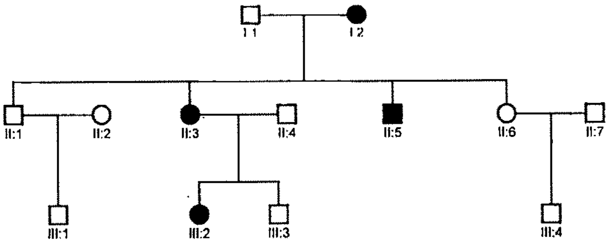

Fig 4. Pedigree of WS type II family with normal hearing. Square — man, circle — woman, shaded symbol — affected individual.

*Patient Groups*. All of these patients were normal by otologic examination. There was no significant history of exposure to other known causes of hearing loss, such as noise exposure and ototoxic drugs. All the patients in group 1 were found bilaterally to have air conduction thresholds of no poorer than 20 dB hearing level at octave intervals from 0.25 to 8 kHz. Normal middle ear function and acoustic reflex thresholds were identified bilaterally in all patients. In patient group 2, there were 2 patients with bilateral low-frequency hearing losses (Fig 2A,B), and the remaining 1 had unilateral low-frequency hearing loss (Fig 2C). Normal middle ear function was found in all 3 patients.

Seven patients from 4 families (12 ears) were found to have wide notches (Fig 3). In 1 type II family (Fig 4), all affected had a similar wide notch (Fig 5), and 2 unaffected family members (II-1 and II-6) had no such notches. Three patients (5 ears) from 2 families with low-frequency hearing loss were tested with the ILO92 to compare the patients' DP-grams with their pure tone audiograms. The results showed that all DP-grams also had an ascending shape, consistent with those found on their pure tone audiograms (Fig 2).

## DISCUSSION

The reason for hearing impairment in WS individuals is not clear. The lack of information on the

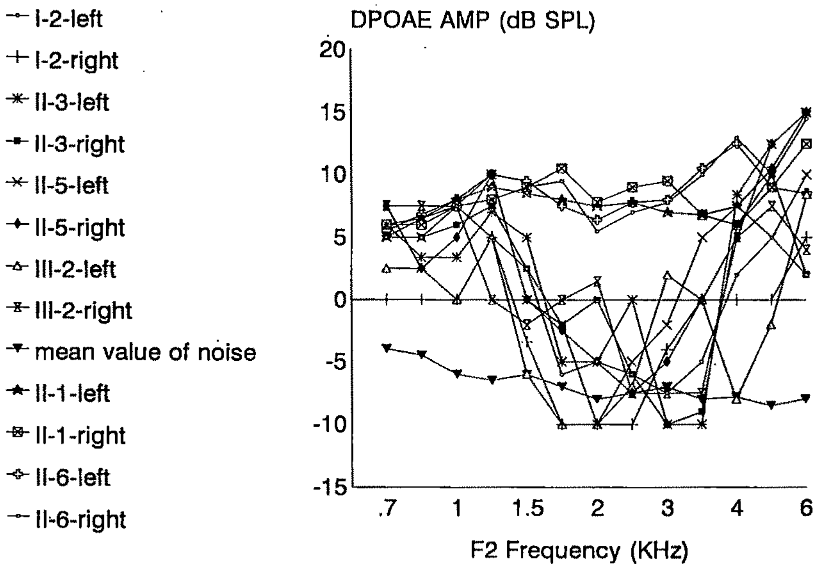

Fig 5. DP-grams of 6 individuals from WS type II family of Fig 4. L1 = L2 = 70 dB SPL.

inner ear abnormalities in families with identified PAX3 mutations and MITF mutations makes correlations between the molecular pathology and its effects on auditory function difficult. It is not clear whether or not the WS genes in individuals with normal audiograms have deleterious effects on the inner ear not detected by traditional audiometry. Liu[9] has studied 26 WS patients with normal hearing using Audioscan audiometry. No notches were found among the patients. It may be that the Audioscan was insensitive to subclinical aberrations occurring in normal-hearing carriers of WS genes.

In this study, notches in the DPOAE audiogram were found in 10% of control subjects. This is compatible with the estimate of some studies that 1 in 8 of the general population are carriers of recessive genes for hearing loss,[26,27] but it could have been due to chance occurrences in a relatively limited control population.

Among 8 patients with WS, 7 (87.5%) were identified to have a notch in their DP-grams in the frequency range 1,000 to 3,000 Hz, which was significantly higher than that found in the control group. Although these results are based on relatively small numbers of individuals with WS, the DPOAE results do seem to indicate clear subclinical pathologic changes.

Notches in the DP-gram of normal-hearing individuals may represent a physical phenomenon or may indicate subclinical pathologic changes. When two waves that are not exactly in phase combine, the resultant wave will have a different amplitude and different phase angle from either of the component waves. These notches may be simply a manifestation of the physical principles of waves from different sources adding together. Another possible source of a notch could be a small area of damage on the cochlea that fails to produce a DP of similar amplitude to the area immediately adjacent to the lesion. It is believed that the cochlea performs, not as independent cells, but in "channels" approximately one-third-octave wide. Therefore, damage to the cochlea would be expected to give rise to a wider notch than those seen in a normal ear.

We have found that the examined normal-hearing carriers of the genes for WS showed changes in their DP-grams that may represent a subclinical disorder. This finding is important, not only for improving diagnosis (particularly in patients with type II), but also for research into audiologic features of WS. This would be of particular value in the diagnosis of ambiguous cases and in genetic counseling. From the present study, all type II patients (100% ears) had the notches, but 2 type I patients (33% ears) had notches. This may reflect the fact that damage to the auditory organ in WS is more common in type II than type I. Due to the small number of cases studied in this investigation, it is hard to identify the difference between type I and type II genes. It is possible that it would be present if more cases had been tested.

The implication of the frequency-specific properties of DPOAEs is that each part of the cochlea can be detected by looking at the amplitude on the DP-gram. Defining such a predictive relationship could be of great clinical significance. Many studies have indeed shown that there does appear to be a good correlation between DPOAE amplitude and the pure tone hearing thresholds; ie, that the amplitude of the DPOAE decreases when hearing loss occurs.[14,15,28] Most of these data concerning the relationship between DPOAEs and hearing loss have been in the form of individual reports. More recently, having analyzed DP emission data and pure tone thresholds in 229 normal-hearing and hearing-impaired ears, Kimberley et al[29] suggested that DP emission measures could be used to reliably predict pure tone threshold status in a large population of ears with different hearing configurations.

Low-frequency sensorineural hearing loss has more than 15 various causes.[12] It is one of the main hearing impairment patterns in patients with WS.[9] There are few reports on the relationships between DPOAEs and low-frequency hearing loss. However, defining such predictive relationships could be of great clinical significance. If a strong correlation exists between the DP-gram and the pure tone audiogram in patients with low-frequency hearing loss, then the DP-gram alone may provide an early objective test for infants in a family with low-frequency hearing loss.

In the present investigation, all tested patients with low-frequency loss showed a consistent pattern of activity between the individual DP-gram and their hearing loss, but the consistent relationship is not quantitative. These results suggest that the deficiency in the functioning of the inner ear indicated by the reduced DPOAEs may make a significant contribution to the genetically caused, low-frequency hearing loss displayed by these patients. This suggests that the DP-gram may be a better predictor of low-frequency hearing loss than ABR using clicks, which is high-frequency–biased (2 to 4 kHz). This will be of value in genetic counseling and objective evaluation of children with suspected low-frequency hearing loss, and will enhance service provision for affected children. In hearing aid fitting for children, it is important to detect low-frequency hearing loss to avoid underamplification of the lower frequencies; only high-frequency information is obtained through ABR. Clearly, the preliminary results were on small numbers, and it is important to determine the validity of these claims in a larger group.

A robust emitted response not only is associated with a healthy outer hair cell system, but also indicates that the middle ear apparatus is in good condition. Thus, the absence of, or appreciable reduction in, emission levels may not necessarily reflect deficient cochlear generators. The presence of abnormal emissions in the ears of children and adults must be interpreted cautiously, and necessarily in combination with the results of immittance testing.

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*Ann Otol Rhinol Laryngol* 106:1997

# SALIVARY GLAND DOUBLE TUMOR: SYNCHRONOUS IPSILATERAL PLEOMORPHIC ADENOMA AND ACINIC CELL CARCINOMA OF THE PAROTID GLAND

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A 44-year-old woman underwent a partial parotidectomy for a slowly enlarging parotid mass diagnosed by fine needle aspiration biopsy as a pleomorphic adenoma. Though macroscopically recognized to be composed of two nodules, differing to some extent from one another, the mass appeared to constitute a single tumor. The microscopic examination disclosed two disparate neoplasms, which were separated from each other by a thin fibrous band. The larger of the two nodules was a pleomorphic adenoma and the smaller one an acinic cell carcinoma. The metachronous — and, even more, the synchronous — occurrence of two histologically different tumors in one major salivary gland is an exceptional and probably coincidental event.

KEY WORDS — acinic cell carcinoma, pleomorphic adenoma, salivary gland double tumor.

## INTRODUCTION

Benign and malignant epithelial tumors of the parotid gland are commonplace in the everyday practice of a surgically active otolaryngology department. Yet the coexistence of neoplasms of different histologic types is an extraordinary event. Metachronous or bilateral tumors are more prevalent than synchronous or ipsilateral tumors. The purpose of this report is to describe the case of a patient with a pleomorphic adenoma and an acinic cell carcinoma clinically and grossly presenting as a solitary parotid mass.

## CASE REPORT

A 44-year-old woman was hospitalized for the treatment of a slowly enlarging, otherwise asymptomatic swelling of the left parotid region. The general physical and otolaryngological examinations were unrewarding. The results of routine laboratory tests were within the normal range. A nontender, elastic, roundish lump, estimated to measure 2 × 3 cm, was palpated in front of the tragus. It extended backward of the mandibular angle, where a second small nodule was thought to be present. Fine needle aspiration biopsy disclosed clusters and sheets of small, solidly packed, monotonous, benign-appearing cells with a gradual transition from the sheets to a loosely arranged myxoid stroma comprising abundant fibrillary ground substance and isolated cells. With the diagnosis of pleomorphic adenoma, the patient underwent partial parotidectomy. The tumor was enclosed within a thin pseudocapsule, which was adherent to the facial nerve. Three and a half years postoperatively, the patient is in good health without recurrent disease and with well-preserved facial nerve function.

The operation specimen measured 4.5 × 3.5 × 2.5 cm. It consisted of glandular parenchyma surrounded by some fat tissue. The cut sections revealed a single mass comprising of two closely adjoining nodules, measuring 2.1 and 0.9 cm in their longest diameter. The larger of the two nodules was sharply demarcated, polycyclic, mucoid, whitish gray, and firmly elastic. The smaller nodule was poorly outlined, white, and soft. Histologic examination disclosed two distinct tumors: a larger pleomorphic adenoma and a smaller acinic cell carcinoma. The neoplasms were separated from each other by a thin fibrous band containing atrophic acini and ducts (Fig 1).

The pleomorphic adenoma was surrounded by a delicate fibrous pseudocapsule. It was composed of scant, small, fusiform or stellate cells and epithelial-type formations set in a greatly dominant basophilic myxomatous background. The epithelial formations consisted of slender cords and glandlike or branching ductlike structures, the lumina of which contained eosinophilic secretory material. Cell pleomorphism and mitotic figures were not evident. Immunohistologically, the cells were mostly decorated by antibodies to cytokeratin, S-100 protein, and actin, while the glandular and tubular luminal cells reacted also with antibodies to epithelial membrane antigen.

The acinic cell carcinoma bordered on the pleomorphic adenoma on one of its aspects, being else-

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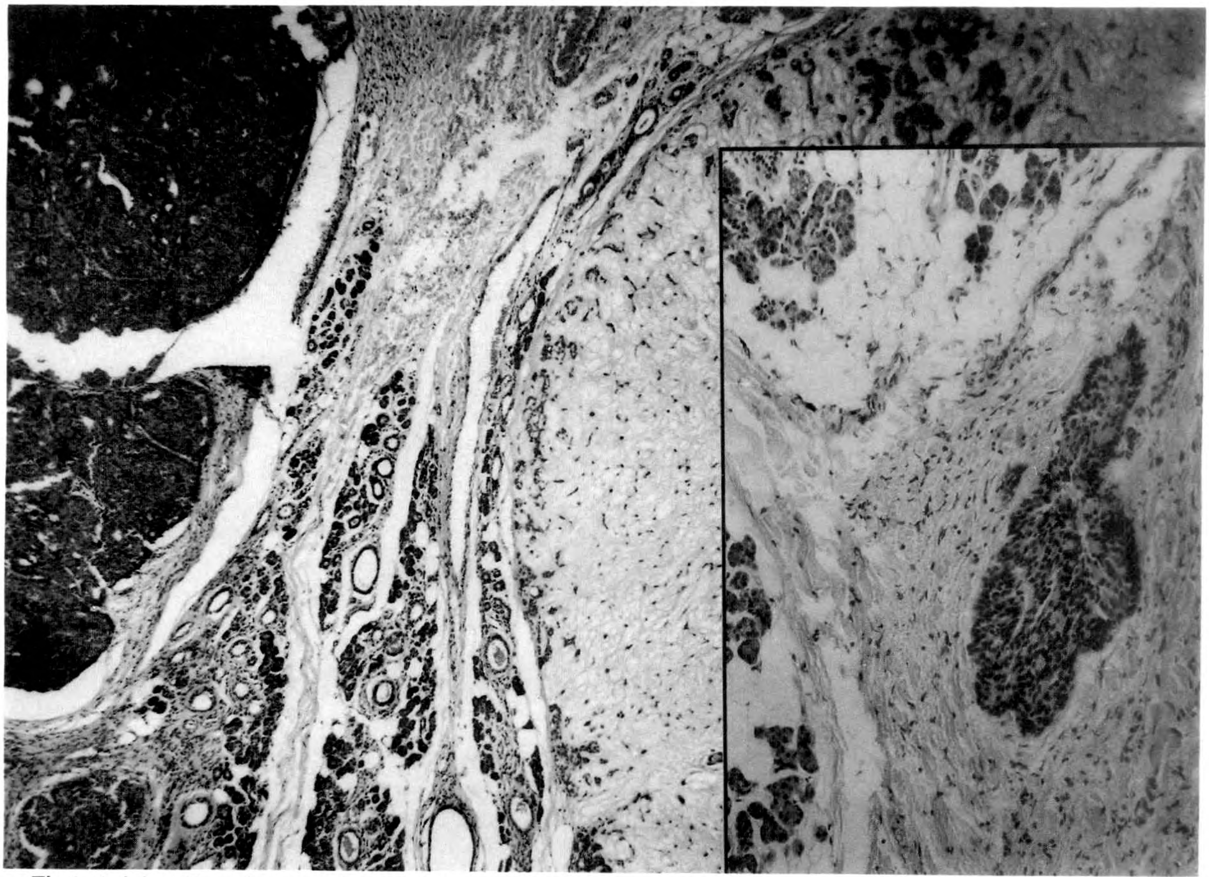

Fig 1. Acinic cell carcinoma (left) and juxtaposed pleomorphic adenoma (right), separated from one another by loosely textured fibrous tissue containing atrophic acini and ducts (H & E, original ×20). Inset) Carcinomatous focus at distance from main tumor mass in between parotid parenchymatous lobules (H & E, original ×80).

where surrounded by fibro-fatty tissue. It profusely infiltrated the fibrous tissue in between the parenchymatous lobules (Fig 1, inset). It was of the mixed solid and microcystic type.[1,2] The variously sized and shaped, partly confluent lobules were encircled by thin, densely textured, collagenous septa, which contained rare atrophic acini. The lobules were composed of closely packed, medium-sized, polyhedral, finely granular, eosinophilic, and faintly periodic acid–Schiff–positive cells. The solid sheets were interrupted by round to irregular lumina that were lined by slightly larger, low columnar cells. The round to oval nuclei were vesicular or finely stippled, with one, and rarely two, small but distinct nucleoli (Fig 2). The lobules were incompletely subdivided into smaller units by thin, collagen and reticulin fiber–holding strands. Round, hyaline, eosinophilic, and periodic acid–Schiff–reactive, 2- to 15-μm globules were randomly scattered in between the cells. Mitotic figures were scarce, the mitotic index being less than 2 per 10 high-power fields. While only some cells were S-100 protein–immunoreactive, all the cells arranged in solid formations were weakly decorated, and those enclosing upon lumina were briskly decorated, by anti-cytokeratin (Fig 2) as well as anti–epithelial membrane antigen antibodies.

## DISCUSSION

Unexpected as it was, the patient's double tumor was not recognized at the time of either clinical evaluation or gross pathologic examination of the operation specimen. With the wisdom of hindsight, we see that attention should have been paid to the unusual presentation. Clinically, the parotid lump was palpated in front of the tragus and extended backward of the mandibular angle, where a second small nodule was thought to be present. Macroscopically, the mass was perceived to be composed of two adjoining portions that texturally differed from each other. Only the larger and superficially located pleomorphic adenoma having been aspirated, the fine needle biopsy disclosed cytologic features of a benign tumor. The operation was planned accordingly, and the presence of the second, malignant neoplasm came as a surprise.

Billroth and von Winiwater are credited as the first to describe double tumors.[3] Excluded by definition are multiple tumors in the framework of hereditary syndromes, multiple tumors of identical histologic

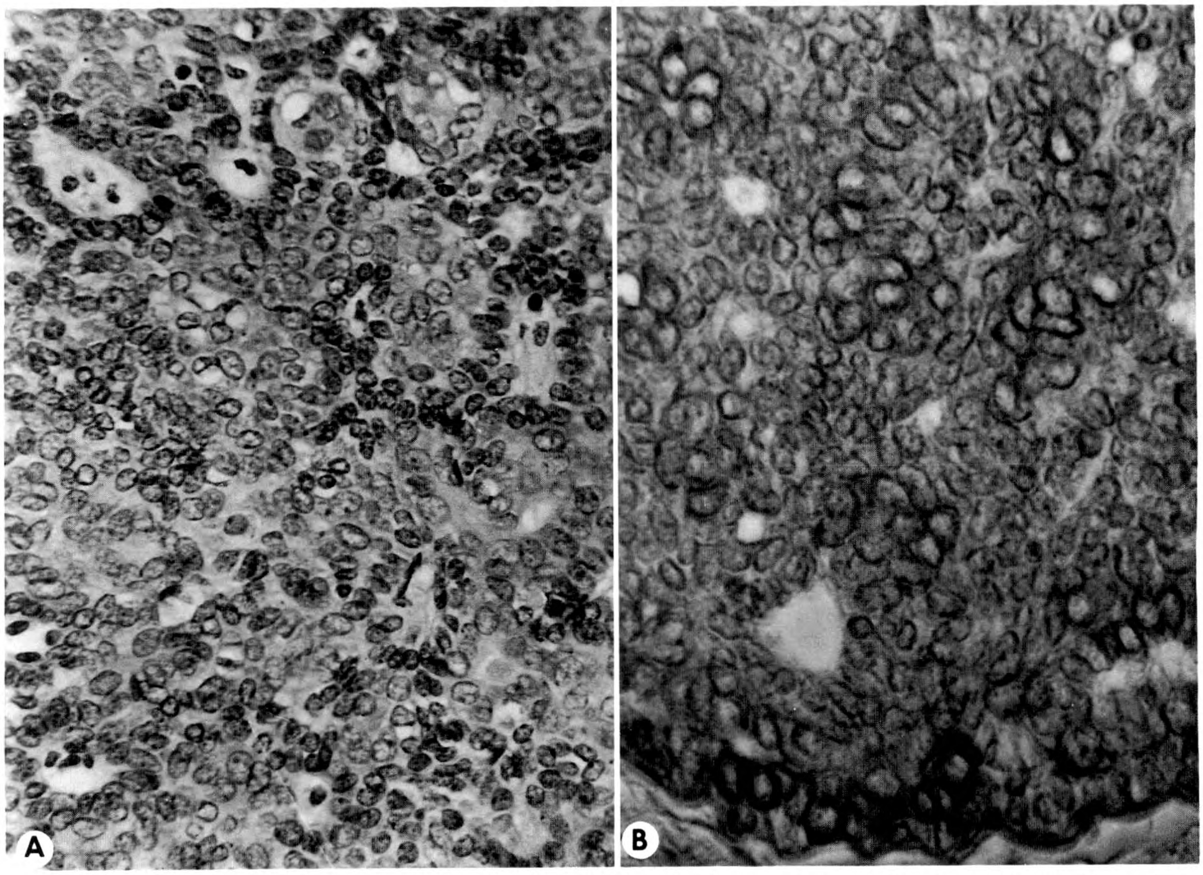

Fig 2. Original ×320. A) Acinic cell carcinoma showing mixed solid and microcystic growth pattern (H & E). Cytoplasm of closely packed, medium-sized, polyhedral cells is faintly granular. Nuclei are round to oval, vesicular, and finely stippled with one, or rarely two, small nucleoli. B) Tumor cells in solidly patterned areas are weakly immunoreactive, and those enclosing on lumina or at lobular perimeters are briskly decorated with epithelial cell markers. Section was processed by avidin-biotin immunostaining technique for detection of cytokeratin.

genre, focal malignant transformation within a benign tumor, eg, carcinoma ex pleomorphic adenoma,[4] and metastatic deposits in an organ harboring another primary neoplasm. The most widely accepted definition of a double tumor is the metachronous or synchronous occurrence in one and the same organ of two neoplasms deviating from one another in their histologic composition.[5]

Pleomorphic adenomas account for the vast majority of salivary gland tumors, and close to two thirds are located in the parotid gland. Predictably, most double tumors involve the parotid gland and constitute pleomorphic adenomas associated with one of the less common varieties of salivary gland neoplasms.[6] In addition to two epithelial neoplasms, two mesenchymal tumors or an epithelial tumor and a mesenchymal neoplasm or lymphoma have also been reported to have arisen in one and the same salivary gland.[7,8] In contrast to these uncommon, genuine double tumors, affecting just 0.4% of patients with salivary gland neoplastic diseases, metachronous or synchronous tumors of different ipsilateral or contralateral glands are not infrequently encountered, occurring in 1.3% of this patient population.[7,9] In view of the relatively low incidence of salivary gland malignancies, two histologically disparate cancers make up the rarest combination of a double tumor. More often than not, two or more tumors in one and the same salivary gland are of a histologically identical nature, in which case they should be referred to as "multiple" rather than double tumors.[10-12]

Taxonomically disparate neoplasms arising either concurrently or sequentially in a single gland most likely constitute an incidental event. Leventon et al[13] reported on a patient with a malignant oncocytoma diagnosed 9 years after excision of a pleomorphic adenoma of the ipsilateral parotid gland. A 68-year-old patient described by George et al[14] presented with a terminal duct carcinoma 8 years after excision of a pleomorphic adenoma of the ipsilateral parotid gland. In the experience of Gnepp et al,[15] the most commonly seen combination is a Warthin's tumor and carcinoma, while pleomorphic adenomas are infrequently associated with cancers.

Unless the two components of the double tumor are grossly separated from each other by sizable normal salivary gland parenchyma or a thick fibrotic tract, their recognition as two distinct types of neoplastic disease is an exercise in the unachievable at the time of gross inspection. Similar to our experience, Gaynor and Hershberg[16] identified on gross examination a single, 3.5-cm mass that microscopically turned out to consist of a large pleomorphic adenoma bordering on, but separate from, a small Warthin's tumor. Similarly, in the two cases reported by Bab and Ulmansky,[17] the macroscopically single tumorous masses proved histologically to be composed of an oncocytoma associated with a carcinoma ex pleomorphic adenoma and a sebaceous cell adenoma associated with an adenoid cystic carcinoma, respectively.

Notwithstanding the marginal excision of the acinic cell carcinoma, our patient's prognosis was expected to be favorable. In fact, she is free of recurrent disease at the 42-month follow-up examination. It is improbable that a high index of awareness and attention to subtle clinical and macroscopic findings would allow for suspecting the presence of most patients' salivary gland double tumors prior to histologic study. Aside from the fact that our observation is representative of the patient population with salivary gland double tumors, this nosologic entity may be relegated to medical oddities of no special clinical or prognostic importance.

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## FIFTH INTERNATIONAL COCHLEAR IMPLANT CONFERENCE

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*Ann Otol Rhinol Laryngol* 106:1997

# SIGNAL TRANSDUCTION PATHWAYS IN MODULATION OF CILIARY BEAT FREQUENCY BY METHACHOLINE

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The release of endogenous neurotransmitters plays an important role in the airway mucosal defense system. We studied the in vitro effect of methacholine, a β-methyl ester of acetylcholine, on the ciliary beat frequency (CBF) of human adenoid explants and its mechanism of action. Tissue explants were cultured at 35°C and covered with 1.0 mL of culture medium: minimum essential Eagle's medium (MEM) containing L-arginine ($1.2 \times 10^{-3}$ mol/L). Methacholine was added to the cultured tissue at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L. The CBF was determined by phase contrast microscopy and microphotometry. Methacholine increased CBF in a dose-dependent manner with a maximum increase of 23.0% ± 1.8% ($p < .001$). Atropine ($10^{-6}$ mol/L) significantly inhibited the ciliostimulatory effects of methacholine ($p < .0007$). The role of endogenous prostaglandins in methacholine-induced ciliostimulation was determined by treating specimens with a cyclooxygenase inhibitor (diclofenac sodium). Diclofenac ($10^{-6}$ mol/L) significantly inhibited the ciliostimulatory effects of methacholine ($p < .0007$). To determine if nitric oxide (NO) acts as an intermediary in ciliostimulation by methacholine, endogenous NO production was inhibited by treating specimens with an L-arginine analog, $N^G$-nitro-L-arginine methyl ester (L-NAME), prior to addition of methacholine. L-NAME ($10^{-6}$ mol/L) inhibited the effects of methacholine in L-arginine–free MEM ($p < .008$), and this inhibition was reversed by L-arginine ($10^{-3}$ mol/L). To further examine the actions of NO in methacholine-induced ciliostimulation, a cyclic guanosine 3'5'-monophosphate (cGMP) kinase inhibitor (KT-5823) was used, prior to the addition of methacholine. KT-5823 ($10^{-6}$ mol/L) significantly inhibited the effects of methacholine ($p < .0001$). Ciliostimulation by methacholine in human upper airway mucosa involves both prostaglandin and NO second messengers and activation of a cGMP-dependent kinase.

KEY WORDS — ciliary beat frequency, methacholine, signal transduction pathways.

## INTRODUCTION

The parasympathetic nervous system is believed to play an important role in the airway defense system. Methacholine, a β-methyl ester of acetylcholine, accelerates the mucociliary wave frequency, and this effect can be blocked by the muscarinic receptor antagonist atropine.[1] Other studies have reported contradictory effects on mucociliary transport by blocking muscarinic receptors.[2]

Various signal transduction mechanisms in acetylcholine-mediated physiological actions have been reported. Acetylcholine applied to endothelium stimulates the production of nitric oxide (NO) and a prostaglandin.[3] Acetylcholine inhibits basal adenosine 3'5'-cyclic monophosphate (cAMP) formation and stimulates inositol monophosphate accumulation in endothelial and smooth muscle cells of rabbit aorta.[4] Acetylcholine applied to corpus cavernosum tissue results in the release of prostaglandins and NO, and NO directly stimulates soluble guanylate cyclase, increasing cyclic guanosine 3'5'-monophosphate (cGMP).[5] Muscarinic agonists have similar NO- and cGMP-dependent actions in thyroid[6] and pancreatic acinar cells.[7]

The aims of the present study were to demonstrate the in vitro effects of methacholine and the muscarinic antagonist atropine on the ciliary beat frequency (CBF) of ciliated human adenoid explants; to determine the role of endogenous prostaglandins and NO second messengers in signal transduction; and to investigate the role of cGMP and cAMP in the modulation of methacholine-induced ciliostimulation. Determining the possible interactions of methacholine in nasal mucosa may contribute to the understanding of the physiological and pathological roles of acetylcholine.

## MATERIALS AND METHODS

*Tissue Preparation.* Ciliary activity was studied in vitro with human adenoid explants. Human adenoid tissue was obtained in the operating room from patients undergoing surgery for nasal obstruction. The tissue was immersed in Medium 199 (Sigma, St Louis, Mo) and transported to the laboratory. Specimens were cut into 3 × 3 × 2-mm pieces and washed three times in clean Eagle's minimum essential medium (MEM; Sigma) to remove blood and debris. Specimens were screened for viable ciliated edges. Those pieces with ciliated edges were placed onto a sterile 35-mm plastic culture dish, which had been coated with type I collagen (Sigma) diluted 1:10 in

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MEM and sterilized overnight under ultraviolet light. The ciliated specimens were covered with 1.0 mL of culture medium MEM containing L-arginine ($1.2 \times 10^{-3}$ mol/L) and kept in a 95% humidified 5% carbon dioxide incubator at 35°C until used for experiments. The culture medium in each dish was replaced every 2 days. At the time of experiment, specimens were examined under the phase-contrast microscope and screened for bacterial or fungal contaminants. Only explants free of any bacterial or fungal contamination were used. Specimens were used for experiments only once every 24 hours to allow the tissue to return to equilibrium. A minimum of five trials were done to establish the average change in CBF for each concentration of the various experiments.

*Solutions.* We used MEM as the substrate medium in the preparation of all experimental solutions. It was supplemented with 160.0 U/mL penicillin and 0.16 mg/mL streptomycin (Sigma). Experimental solutions were made with 1% albumin (Sigma) in MEM containing L-arginine ($1.2 \times 10^{-3}$ mol/L) and adding methacholine chloride, diclofenac sodium, or atropine sulfate (all from Sigma). KT-5823 and H-89 (Calbiochem, San Diego, Calif) were initially dissolved in dimethyl sulfoxide (DMSO; Sigma) to a concentration of $10^{-4}$ mol/L. These solutions were diluted in MEM to their final experimental concentration of $10^{-6}$ mol/L. The Select Amine Kit (Gibco, Grand Island, NY) was used to make arginine-free MEM. Methacholine was used in concentrations of $10^{-6}$, $10^{-8}$, and $10^{-10}$ mol/L; diclofenac, atropine, and $N^G$-nitro-L-arginine methyl ester (L-NAME) at $10^{-6}$ mol/L; and L-arginine at $10^{-3}$ mol/L. Stock solutions were made and kept frozen until the day of the experiment. Minimum essential medium was supplemented with antibiotics as above and used as culture medium. Medium 199 was used as transport medium.

*Analysis of Ciliary Beat Frequency.* The specimens were placed on the heated stage of an inverted phase-contrast microscope (Nikon 8328) and allowed to equilibrate for 15 minutes. The stage was heated to 33°C to approximate in vivo temperatures. The cilia were viewed at 400× and oriented to interrupt the passage of light through a 0.2-mm slit in the diaphragm of the attached photometer (Nikon P-1). The fluctuating light produced by the ciliary beats was processed into an electrical analog signal. The signal was amplified, filtered through 5-Hz and 30-Hz filters, and digitized at 200 Hz. By means of ASYST software (Keithley), a fast Fourier transform was performed on the signal to determine modal CBF in hertz. Because there was some variability of baseline CBF, the response to stimulation was reported as percent change in CBF from the baseline level. All values were reported as mean ± SEM. Statistical comparisons were made with Student's *t*-test, with a p value of less than .05 being considered statistically significant.

*Effects of Methacholine.* After equilibration, baseline CBF of a specimen was determined by averaging 20 successive measurements obtained from the same ciliated site. The medium covering the specimen was removed and replaced with 1.0 mL of MEM containing methacholine. The same site on the specimen was relocated, and the first CBF measurement was made within 2 minutes. Fifty measurements were averaged to determine the effect of methacholine alone at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L.

*Effects of Atropine.* Baseline CBF was determined by averaging 20 successive measurements. The MEM covering the specimen was removed and replaced with 1.0 mL of MEM containing atropine. Thirty CBF measurements were performed over 15 minutes to determine the effect of atropine alone on baseline CBF. After 15 minutes, the atropine was removed and methacholine was added. Thirty subsequent measurements were obtained at the same site on each specimen to determine the response to methacholine after muscarinic receptor blockade by atropine.

*Effects of Prostaglandins.* Experiments investigating the role of endogenous prostaglandins were performed after determining baseline CBF. The MEM covering the specimen was removed and replaced with 1.0 mL of MEM containing diclofenac, a cyclooxygenase inhibitor. Thirty CBF measurements were made over 15 minutes to determine the effect of diclofenac alone on baseline CBF. After 15 minutes, the diclofenac was removed and methacholine was added. Thirty subsequent measurements were obtained for the given site on each specimen to determine if prostaglandin synthesis was required for the activity of methacholine.

*Effects of Nitric Oxide.* Experiments investigating the role of endogenous NO were performed by using L-arginine–free MEM to inhibit the endogenous synthesis of NO. The MEM containing L-arginine was removed from the specimens and replaced with 1.0 mL of L-arginine–free MEM. Specimens were used for experiments after being cultured in L-arginine–free MEM for 24 hours. Baseline CBF was determined in the arginine-free MEM. The arginine-free MEM was replaced with arginine-free MEM containing methacholine. Fifty measurements were averaged to determine the effect of methacholine alone at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L.

Additional experiments investigating the synthe-

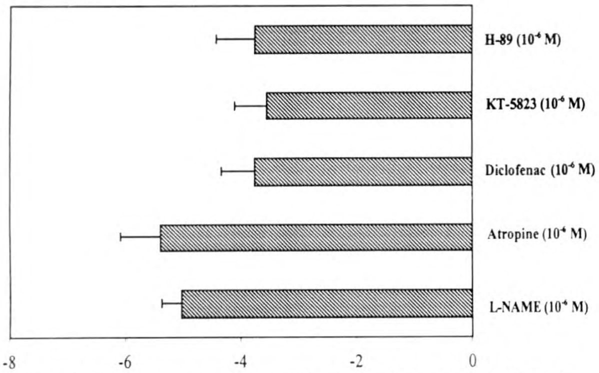

Fig 1. Changes in baseline ciliary beat frequency (CBF) in percent after treatment with inhibitors: H-89, cAMP inhibitor; KT-5823, cGMP inhibitor; diclofen, cyclooxygenase inhibitor; atropine, muscarinic receptor antagonist; and L-NAME, nitric oxide synthase antagonist. All of these changes were statistically significant ($p < .001$).

sis of endogenous NO were performed by using L-arginine analogs to further inhibit endogenous production of NO. After determination of baseline CBF in L-arginine–free MEM, L-arginine–free MEM containing L-NAME was added for 15 minutes and 30 CBF measurements were made to determine the effects of further NO inhibition on baseline CBF. After 15 minutes, the L-NAME was removed and L-arginine–free MEM containing methacholine and L-NAME was added. Thirty subsequent CBF measurements were made to determine the effect of methacholine in the presence of NO inhibition. After 15 minutes, this solution was replaced with MEM containing methacholine and L-arginine to determine if the inhibitory effect of the L-arginine analog could be reversed.

*Effects of cGMP and cAMP*. Experiments investigating the role of cGMP kinase were performed by using L-arginine–supplemented MEM ($1.2 \times 10^{-3}$ mol/L) to avoid inhibition of endogenous NO production. Baseline CBF was determined by averaging 20 successive measurements. The MEM covering the specimen was removed and replaced with 1.0 mL of MEM containing KT-5823. Thirty CBF measurements were made over 15 minutes to determine the effects of cGMP kinase inhibition on baseline CBF. After 15 minutes, the KT-5823 was removed and MEM containing methacholine and KT-5823 was added. Thirty subsequent CBF measurements were made to determine the effect of methacholine in the presence of cGMP kinase inhibition. The same experimental methods were performed with H-89 used to investigate the effects of cAMP kinase inhibition on ciliostimulation.

## RESULTS

*Baseline CBF*. The ciliated adenoid explants were maintained in culture for up to 18 days. The mean baseline CBF (mean ± SEM) was 7.64 ± 1.02 Hz. Both MEM alone and DMSO (10%) in MEM were used as negative controls. Neither of these produced a significant change in the CBF. Small decreases in baseline CBF were produced by L-NAME ($10^{-6}$ mol/L), atropine ($10^{-6}$ mol/L), diclofenac ($10^{-6}$ mol/L), KT-5823 ($10^{-6}$ mol/L), and H-89 ($10^{-6}$ mol/L) (Fig 1).

*Effects of Methacholine*. Methacholine alone ($10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L) in arginine-supplemented MEM increased the CBF by 8.06% ± 1.42%, 12.39% ± 1.63%, and 23.03% ± 1.77%. These increases were statistically significant by Student's paired *t*-test with $p < .001$ (Fig 2A).

*Effects of Atropine*. After treatment with $10^{-6}$ mol/L atropine, the ciliary responses to methacholine alone at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were −2.95% ± 0.64%, 1.74% ± 1.33%, and 8.59% ± 0.76%. The inhibition of methacholine-induced ciliostimulation by atropine was statistically significant ($p < .0007$ by unpaired *t*-test), as shown in Fig 2A.

*Effects of Prostaglandins*. After treatment with $10^{-6}$ mol/L diclofenac, the ciliary responses to methacholine at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were −4.13% ± 0.65%, −4.09% ± 1.30%, and −4.14% ± 0.36%. The inhibition of methacholine-induced ciliostimulation by diclofenac was statistically significant ($p < .0007$ by unpaired *t*-test), as shown in Fig 2A.

*Effects of Nitric Oxide*. Methacholine alone in L-arginine–free MEM at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L increased CBF by 3.25% ± 0.27%, 5.03% ± 0.55%, and 8.55% ± 0.95% (Fig 2B). These increases were statistically significant ($p < .001$). The inhibition of methacholine-induced ciliostimulation in L-arginine–free MEM was statistically significant ($p < .008$ by unpaired *t*-test) when compared to methacholine-induced ciliostimulation in arginine-supplemented MEM.

After treatment with L-NAME ($10^{-6}$ mol/L) to further inhibit endogenous NO production, the ciliary responses to methacholine at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were −1.28% ± 0.44%, 0.43% ± 0.48%, and 1.64% ± 0.13%. The inhibition of methacholine-induced ciliostimulation by L-NAME was statistically significant ($p < .0001$ by unpaired *t*-test). The L-arginine ($10^{-3}$ mol/L) reversed this inhibition by L-NAME, resulting in ciliary responses of 1.65% ± 0.59%, 5.58% ± 0.79%, and 7.47% ± 0.23% (Fig 2C).

*Effects of cGMP and cAMP*. After treatment with the cGMP kinase inhibitor, KT-5823 ($10^{-6}$ mol/L),

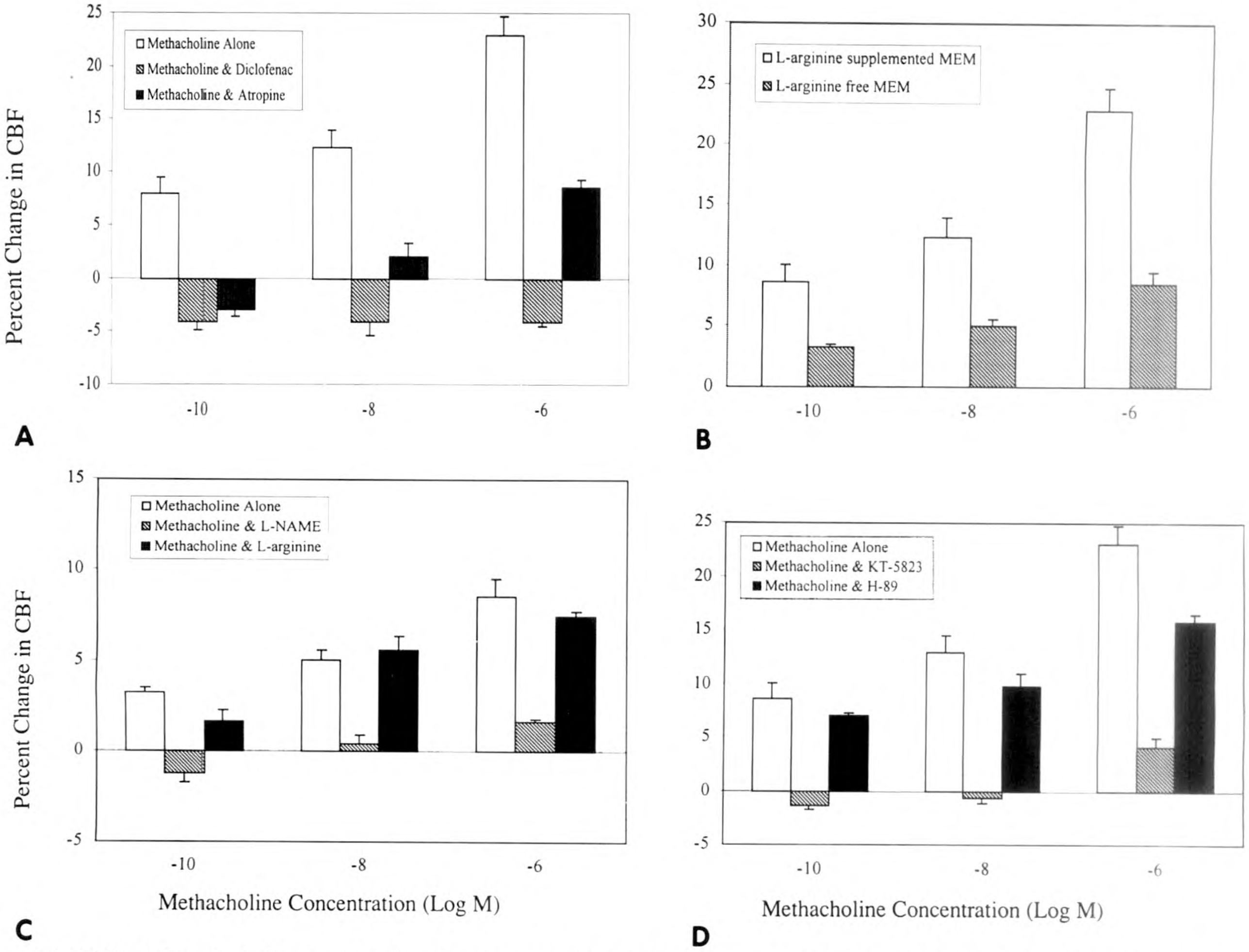

Fig 2. Effect of methacholine on ciliary beat frequency after blockade of muscarine, prostaglandin, nitric oxide, cGMP, and cAMP pathways. A) Methacholine alone in arginine-supplemented Eagle's minimum essential medium (MEM; open bars) increased CBF in dose-dependent manner. Increases were significant at $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L ($p < .001$). Atropine (slashed bars) significantly inhibited ciliostimulation of methacholine ($p < .0007$). Diclofenac (darkened bars) significantly inhibited ciliostimulation of methacholine ($p < .0007$). B) Methacholine in arginine-supplemented MEM (open bars) increased CBF in dose-dependent manner. Increases were significant at all concentrations ($p < .001$). Methacholine in arginine-free MEM (slashed bars) increased CBF in dose-dependent manner. Increases were significant at all concentrations ($p < .001$). Inhibition of methacholine-induced ciliostimulation by arginine-free MEM was significant at all concentrations ($p < .008$). C) Increases in CBF with methacholine alone in L-arginine–free MEM (open bars) at $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were significant ($p < .001$). Ciliostimulation seen with methacholine alone at $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L was significantly inhibited by L-NAME $10^{-6}$ mol/L (slashed bars) ($p < .0001$). Inhibition was reversed by adding L-arginine $10^{-3}$ mol/L (darkened bars). D) Ciliostimulation seen with methacholine alone at $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L (open bars) was significantly inhibited by KT-5823 $10^{-6}$ mol/L (slashed bars) ($p < .0001$). After treatment with H-89 ($10^{-6}$ mol/L), at methacholine concentration of $10^{-6}$ mol/L there was statistically signifi-cant inhibition (darkened bars) ($p < .05$). Inhibition of methacholine effects on CBF by H-89 at methacholine concentrations of $10^{-10}$ and $10^{-8}$ mol/L was not statistically significant ($p = .26$).

the ciliary responses to methacholine at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were −1.38% ± 0.37%, −0.72% ± 0.48%, and 4.12% ± 0.91%. The inhibition of methacholine-induced ciliostimulation by KT-5823 was statistically significant ($p < .0001$). After treatment with the cAMP kinase inhibitor, H-89 ($10^{-6}$ mol/L), the ciliary responses to methacholine at concentrations of $10^{-10}$, $10^{-8}$, and $10^{-6}$ mol/L were 7.00% ± 0.33%, 9.86% ± 1.17%, and 15.80% ± 0.79%. Methacholine at a concentration of $10^{-6}$ mol/L was significantly inhibited by H-89 ($p < .05$). The inhibition of methacholine by H-89 at methacholine concentrations of $10^{-10}$ and $10^{-8}$ mol/L was not statistically significant ($p = .26$), as shown in Fig 2D.

## DISCUSSION

*General Considerations.* Our method of culturing adenoid tissue permitted the study of ciliated epithelium that remained attached to its basement membrane and closely resembled in vivo organization. This method also offered the advantage of long-term investigation, as we maintained ciliated adenoid tissue for up to 21 days. These are advantages not seen with other in vitro methods of examining ciliated

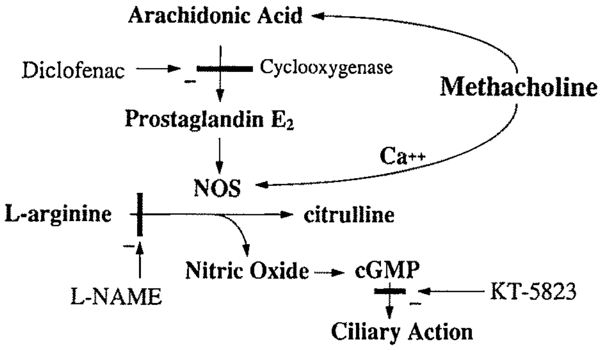

Fig 3. Diagram showing pathways blocked to study signal transduction pathways for methacholine ciliostimulation. Diclofenac is cyclooxygenase inhibitor that blocks synthesis of prostaglandin from arachidonic acid. L-NAME competitively inhibits nitric oxide synthase (NOS) from L-arginine. KT-5823 inhibits cGMP-dependent protein kinase, and thus inhibits action of cGMP on ciliostimulation.

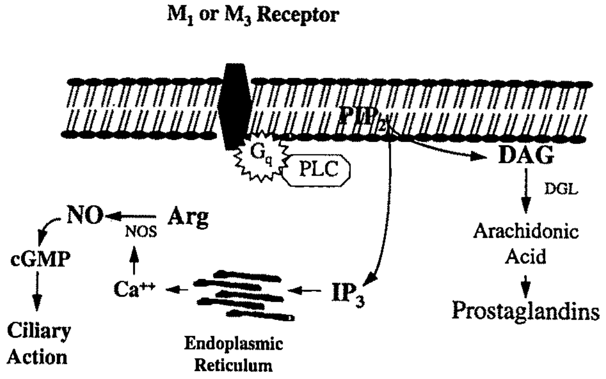

Fig 4. Proposed signal transduction pathway for ciliostimulation by methacholine. M1 and M3 muscarinic receptors are G protein–linked receptors (Gq) that are linked to phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol biphosphate (PIP2) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG can be degraded by diacylglycerol lipase (DGL) to arachidonic acid and further metabolized via cyclooxygenase pathway to prostaglandins. IP3 releases intracellular $Ca^{2+}$ stores from endoplasmic reticulum. Increased intracellular $Ca^{2+}$ can activate nitric oxide (NO) synthase (NOS) to produce NO from L-arginine (Arg). NO stimulates soluble guanylate cyclase to produce cyclic guanosine 3'5'-monophosphate (cGMP).

cells, such as nasal and tracheal brushings, in which the ciliated epithelium is noncohesive and disrupted from its normal architecture. We found our method to be reliable and reproducible. Bacterial or fungal contamination could alter in vivo or in vitro results; however, we identified no contamination in any of our trials.

The observed modal baseline CBF in this study was 7.54 ± 1.02 Hz, which is somewhat lower than what has been reported as basal CBF in other systems. The reason for this difference is not certain, but may be temperature-related. Our studies were performed at 33°C rather than the more common temperature of 35°C. Other physical factors, such as the viscosity of the culture medium, might also have influenced CBF. We do not believe that this difference from previous studies alters the interpretation of the present data. The reliability of the present data is verified by their reproducibility and the consistency of the responses to stimuli.

*Effects of Atropine*. The nonselective muscarinic receptor antagonist atropine significantly blocked methacholine-induced ciliostimulation. Muscarinic acetylcholine receptors are members of a superfamily of at least five subtypes (M1 to M5) that activate signal transduction pathways through their interaction with G proteins.[8,9] These subtypes differ in their tissue distribution, agonist and antagonist binding affinities, and functions. The M1, M3, and M5 subtypes couple to inositol polyphosphate generation, which activates phospholipase C, while the M2 and M4 subtypes inhibit the generation of cAMP.[10] The M1 and M3 muscarinic receptor subtypes have been identified in human nasal and bronchial tissues. The M3 subtype appears to be most predominant, while M2 receptors are absent.[11] It is likely that atropine blockade of the M3 receptor is responsible for the observed effects on CBF.

*Effects of Endogenous Prostaglandins*. Our studies demonstrated that methacholine-induced ciliostimulation was significantly inhibited when the cyclooxygenase pathway was blocked by diclofenac, suggesting that the action of methacholine in stimulation of respiratory cilia requires endogenous prostaglandin generation. Prostaglandin synthesis may be involved in the transduction of the muscarinic acetylcholine receptor signal. The cholinergic response mediated by the activation of M1 and M3 receptors is coupled to Gq proteins, which activate phosphatidylinositol turnover through the enzyme phospholipase C.[8,9] Phospholipase C hydrolyzes phosphatidylinositol 4,5-biphosphate, yielding diacylglycerol and inositol 1,4,5-triphosphate. Diacylglycerol can be degraded by diacylglycerol lipase to arachidonic acid and further metabolized via the cyclooxygenase pathway to prostaglandins.[12,13] Figure 3 illustrates a proposed mechanism for the effects of methacholine-induced ciliostimulation via production of prostaglandins.

Nasal hyperresponsiveness to cholinergic stimulation is seen in patients with atopic disease.[14] Prostaglandins are released during inflammatory diseases of the respiratory tract, such as allergy.[15] It is unknown if the release of prostaglandins is directly related to the stimulation of muscarinic acetylcholine receptors during inflammation. Prostaglandins have been shown to act as second messengers for methacholine in endothelium[3] and corpus cavernosum.[5] No study to date has examined the effect of methacholine on arachidonic acid metabolites in the regulation of respiratory ciliary activity.

*Effects of Endogenous Nitric Oxide and cGMP.* Our study showed that ciliostimulation induced by methacholine was significantly inhibited by both L-arginine–free MEM and the L-arginine analog, L-NAME, which block the endogenous production of NO. This inhibition was reversed by L-arginine ($10^{-3}$ mol/L). Methacholine-induced ciliostimulation was also significantly inhibited by use of a cGMP kinase inhibitor (KT-5823).

Endogenous NO and cGMP synthesis are involved in the transduction of the muscarinic acetylcholine receptor signal. Respiratory tissues contain predominantly $M_3$ receptors and $M_1$ receptors.[11] The muscarinic response mediated by the activation of $M_1$ and $M_3$ receptors is coupled to the $G_q$ protein that activates phospholipase C. Phospholipase C hydrolyzes phosphatidylinositol 4,5-biphosphate, yielding diacylglycerol and inositol 1,4,5-triphosphate. Inositol triphosphate releases $Ca^{2+}$ from intracellular stores. Increased intracellular $Ca^{2+}$ can activate NO synthase, the enzyme that synthesizes NO from L-arginine.[16] Nitric oxide, in turn, activates soluble guanylate cyclase, resulting in the production of cGMP and activation of cGMP kinases (Fig 4).

Nitric oxide is present in the exhaled air of humans,[17] and NO levels increase during the inflammation seen with atopic asthma and rhinitis and decrease in patients with Kartagener's syndrome.[18,19] It is unknown if the release of NO and cGMP is directly related to the stimulation of muscarinic acetylcholine receptors during these inflammatory reactions. Nitric oxide has been shown to act as a second messenger for acetylcholine in endothelium,[3] corpus cavernosum,[5] pancreatic acinar cells,[7] and thyroid cells,[6] and endogenous NO can directly stimulate soluble guanylate cyclase, resulting in production of cGMP.

*Effects of cAMP.* Methacholine-induced ciliostimulation at a concentration of $10^{-6}$ mol/L was significantly inhibited by the cAMP kinase inhibitor H-89. The methacholine effect on ciliary response at concentrations of $10^{-10}$ and $10^{-8}$ mol/L was not significantly inhibited by H-89. Other investigations have shown that $PGE_2$ acts through cAMP and NO second messengers in rat liver macrophages,[20] and that $PGI_2$ analogs can stimulate ciliary activity via endogenous cAMP production.[21] At higher concentrations, methacholine could stimulate the cyclooxygenase pathway, producing prostaglandins. These endogenous prostaglandins could regulate CBF via stimulation of cAMP production. Other studies in our laboratory have shown that agonists that induce the synthesis of cAMP can stimulate ciliary activity (data not yet published). During the muscarinic response, activation of phosphatidylinositol turnover could result in diacylglycerol, phosphokinase C, inositol 1,4,5-triphosphate, arachidonic acid metabolites, $Ca^{2+}$, or NO. Any of these intermediates may have undetermined actions on cAMP production to stimulate CBF. Finally, the cAMP kinase inhibitor (H-89) does have some nonspecific inhibitory effect on cGMP-dependent kinase. This could have resulted in the partial inhibition seen when high concentrations of methacholine were used to stimulate ciliary activity.

*Further Study.* Further investigation using selective muscarinic antagonists is needed to determine the receptor subtypes used in regulation of CBF in ciliated respiratory epithelium. Characterization of muscarinic receptor subtypes in nasal tissue may have very important implications for anticholinergic therapy of diseases such as allergic rhinitis, bronchial asthma, and chronic bronchitis.[22] Highly selective $M_1$ and $M_3$ muscarinic antagonists could have more specific effects on secretory glands than nonselective muscarinic antagonists and result in fewer anticholinergic side effects.

In our study, inhibition of either endogenous prostaglandin or NO production resulted in complete inhibition of ciliostimulation; this finding suggests that these second messenger pathways are not separate and parallel. Endogenous $PGE_2$ has been shown to increase NO synthesis,[20] while in other tissues NO production leads to the release of $PGE_2$.[23] Additional work in our laboratory has shown that $PGE_2$ acts through NO production to stimulate CBF (data not yet published), but the specific prostaglandins induced by methacholine is an area for further study. Prostaglandins may have undetermined effects on inositol 1,4,5-triphosphate release, calcium mobilization, and NO and cGMP formation.

The specific location of the muscarinic receptors and the site of NO production in human respiratory epithelium remains to be determined. Adenoid cultures contain a variety of cell types, including ciliated epithelial cells, goblet cells, vascular cells, submucosal glands, and connective tissue, which may act upon CBF regulation in a paracrine fashion.

Our study showed that ciliostimulation by methacholine in human upper airway mucosa involves prostaglandin, NO, and cGMP second messengers. Pharmacologic modification of acetylcholine release and endogenous prostaglandin and NO synthesis may eventually permit regulation of this important defense mechanism.

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Ann Otol Rhinol Laryngol 106:1997

# EFFECT OF INTRAOPERATIVE ISCHEMIA ON CHONDROGENESIS BY VASCULARIZED AURICULAR PERICHONDRIUM GRAFTED IN THE AIRWAY

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Vascularized perichondrium and periosteum are potentially ideal grafts for airway reconstruction. We quantitatively evaluated the effect of a 2-hour interruption of blood supply on subsequent growth of cartilage from vascularized auricular perichondrium grafted in the rabbit airway. Two pedicled auricular perichondrocutaneous flaps were elevated and used to repair two small anterior airway defects in each animal ($n = 17$). One of the two flaps was randomly clamped for a 2-hour period during the operation, with the other flap serving as a paired control. We found no significant difference in chondrogenesis between the two groups. Our findings support the use of perichondrium and periosteum as free revascularized grafts for airway reconstruction.

KEY WORDS — airway, chondrogenesis, ischemia, vascularized perichondrium.

## INTRODUCTION

Vascularized perichondrium and periosteum possess many qualities of the ideal graft for airway reconstruction. They have a rich vascular supply and are available with minimal morbidity from multiple donor sites. Their pliability at the time of reconstruction allows for contouring and an airtight repair, yet they are ultimately capable of providing rigid support because of their intrinsic chondrogenic and osteogenic potential. Vascularized perichondrium has been quantitatively shown to generate substantially more cartilage than free perichondrium in the airway.[1] Successful reconstruction of a circumferential tracheal defect in a rabbit model using pedicled vascularized perichondrium has recently been performed.[2]

In humans, the likely sources of vascularized tissue for airway reconstruction will be at a site distant from the neck requiring a free microvascular tissue transfer. Despite the growing interest in perichondrium and periosteum as airway grafts, there is very limited data on the potential effect of ischemia associated with a microvascular procedure on chondrogenesis or osteogenesis. Hartig et al[1] reported that temporary clamping of a pedicled perichondrial flap did not alter chondrogenesis, but they could not draw conclusions from their sample size of only four animals. Furthermore, their findings were in conflict with reports that pedicled periosteal flaps uniformly generated rigid bone, while the majority of flaps failed to do so following microvascular transfer to the neck.[3,4] In the present study we quantitatively assessed the effect of a 2-hour intraoperative ischemic insult on chondrogenesis by vascularized perichondrium in the airway.

## MATERIALS AND METHODS

*Animal Model.* The New Zealand White rabbit was chosen as the animal model because it offers a large area of readily accessible auricular perichondrium with an identifiable vascular pedicle. This model permitted paired transfer of dorsal auricular perichondrocutaneous flaps to the neck through a tension-free arc of rotation based on the dorsal auricular vascular pedicle. Seventeen adult rabbits of both sexes weighing 2.5 to 3.5 kg were used.

*Experimental Design.* After anesthesia with xylazine hydrochloride (10 mg/kg intramuscularly [IM]) and ketamine hydrochloride (50 mg/kg IM) and administration of chloramphenicol sodium succinate (50 mg/kg IM), the neck and both ears of each animal were shaved, prepared with Betadine, and draped in an aseptic fashion. A perichondrocutaneous flap was harvested from the dorsal surface of each auricle in a manner described by Hartig et al[1] (Fig 1). The flaps were passed through a subcutaneous tunnel connecting the root of the auricle to a midline neck incision. Two airway defects of 12 to 15 mm$^2$ were created anteriorly: one at the cricothyroid membrane and the other 2 cm inferiorly, on the cervical trachea. One of the flap pedicles was randomly clamped with an

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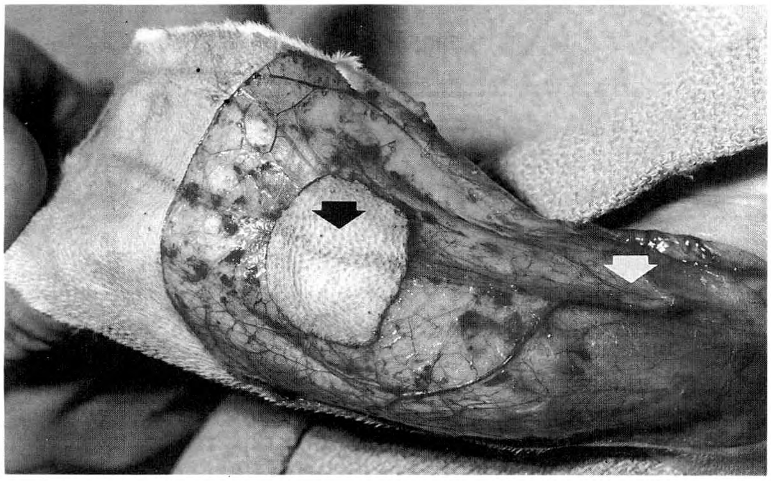

Fig 1. Perichondrocutaneous flap still attached to auricular cartilage depicting skin paddle used for monitoring viability (black arrow) and site of clamping of vascular pedicle (white arrow).

atraumatic vascular clip for a period of 2 hours. Both flaps were used to repair the corresponding airway defects in an airtight fashion with interrupted sutures of 5.0 Dexon. Randomization ensured equal distribution of ischemic flaps from the right versus left ear, as well as to the upper versus lower airway defect. This design allowed each animal to serve as its own control in assessment of the effect of ischemia on chondrogenesis. At closure, both auricular skin paddles were incorporated into the neck incision with interrupted sutures of 4.0 chromic gut. The skin paddles were used for monitoring flap viability for the duration of the experiment (Fig 1). The animals were painlessly sacrificed at 8 weeks and their airways harvested for histologic analysis.

*Complications*. There were no deaths, major airway complications, or signs of vascular compromise of the auricular skin paddle in the neck. There were three immediate postoperative air leaks, all of which were successfully treated with pressure dressings. There were two auricular hematomas, which were treated with incision and drainage. One cervical abscess occurred and was treated with incision and drainage.

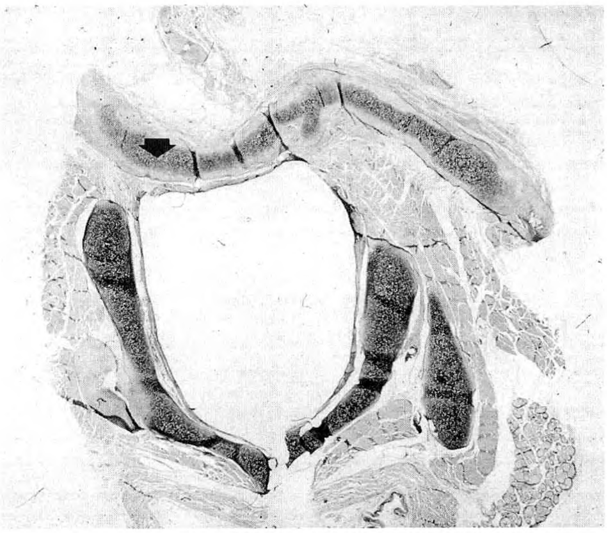

Fig 2. Cross section of grafted portion of trachea showing dark neocartilage (arrow) generated from perichondrial flap.

*Data Analysis*. Each airway was analyzed as two separate specimens that were formalin-fixed, embedded in paraffin, and sectioned in 5-µm-thick sections with a microtome. Twelve slides were prepared from each specimen by taking sections at 0.1-mm intervals along the entire length of the grafted portion of the airway. The slides were stained with hematoxylin and eosin and reviewed by the first author in a blinded fashion (Fig 2). Light microscopic images were digitized onto a computer equipped with image analysis software (MCID-M1, Version 4.20 Rev. 1.0; Imaging Research Inc, Ontario, Canada). With the image on the computer screen, areas of cartilage were easily distinguishable within the specimen. A total of 150 to 200 readings were taken from each airway specimen and combined to obtain the average thickness of each individual specimen.

## RESULTS

Of the 34 flaps, 30 generated measurable amounts of neocartilage. The control flaps had a mean cartilage thickness of 0.337 ± 0.200 mm. The ischemic flaps had a mean cartilage thickness of 0.434 ± 0.106 mm. Analysis of data does not reveal a significant difference in cartilage thickness between the two groups (p = not significant [NS], sign test). By excluding the four animals with flaps exhibiting zero cartilage growth (n = 13), the new thickness mean changes to 0.441 ± 0.063 mm for the control side and 0.459 ± 0.094 mm for the ischemic side (p = NS, paired *t*-test).

The grafts placed in the larynx at the cricothyroid membrane defect generated slightly more cartilage than those placed inferiorly on a tracheal defect

(0.463 ± 0.069 mm versus 0.437 ± 0.088 mm, p = NS). This trend of increased chondrogenesis at the more superior site in the airway was consistent in both the control subgroup (0.460 ± 0.067 mm versus 0.419 ± 0.056 mm, p = NS) and the ischemic subgroup (0.467 ± 0.078 mm versus 0.453 ± 0.111 mm, p = NS).

## DISCUSSION

The major finding of this study is that the chondrogenic potential of vascularized perichondrium in the airway is preserved after a 2-hour interruption of its blood supply. This apparent resistance to temporary ischemia exhibited by perichondrium fulfills an essential prerequisite for its possible free microvascular transfer for airway reconstruction.

In humans, periosteum is the more likely candidate for a vascularized airway graft. Periosteum may be harvested for free microvascular transfer from multiple donor sites, including the iliac fossa, distal humerus, and distal femur,[5] as well as the fibula and scapula. Periosteum and perichondrium are thought to generate cartilage and bone by transformation of mesenchymal cells into mature chondroblasts and osteoblasts.[6] Both require separation from the underlying bone or cartilage to initiate this process, and the rate of the ensuing osteogenesis and chondrogenesis depends largely on the vascular supply.[1,7-9] It is apparent that periosteum and perichondrium share many features in common. Vascularized perichondrium has in fact been shown to generate bone as well as cartilage when placed in a soft tissue environment.[10] Because of these similarities, one may infer that perichondrium and periosteum are also comparable in their resistance to temporary ischemia.

In conclusion, we have demonstrated that perichondrium retains its chondrogenic potential in the airway following a 2-hour ischemic insult. This finding supports the use of free revascularized grafts of perichondrium and periosteum for airway reconstruction.

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*Ann Otol Rhinol Laryngol* 106:1997

# MUCOCILIARY CLEARANCE OF STENTED LARYNGOTRACHEAL TRACT IN GUINEA PIGS IN VIVO

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The purpose of this study was to investigate laryngotracheal mucociliary transport by means of an in vivo guinea pig model with and without a stent. The experimental design involved marking with deep-colored resin powder and utilizing the serial photograph-analyzing method via endoscopic laryngeal videography. Fifteen animals were grouped into two airway conditions: 5 with laryngotracheal stent insertion and 10 without. The mucociliary transit time and mucociliary transport rate were measured in both groups. Significant differences between the two groups were found. In conclusion, stenting preserved and increased the clearance function of the laryngotracheal mucosa in the acute phase.

KEY WORDS — laryngotracheal stenosis, laryngotracheal stent, mucociliary transport.

## INTRODUCTION

In the Department of Otolaryngology, National Taiwan University Hospital, laryngotracheoplasty with stenting is a treatment modality for laryngotracheal stenosis. This modality has been in use since 1977 for a 13-year period with 116 treated patients. Sometimes, it was necessary to place the stent to a level above the vocal cord. Postoperative laryngoscopy showed the upper end of the T-tube above the vocal cord level and near the base of the epiglottis. Our success rate has reached 92%.[1]

Very few investigations exist concerning the mucociliary function with a stent in place. The majority of reports present the findings made after removal of the stent. Nonetheless, the immediate changes in mucociliary status after stent insertion may bear an etiologic relationship to later mucociliary transport and mucosal defense. Furthermore, a similar argument exists for endotracheal intubation — not only for stenting. Thus, the purpose of the present study is to evaluate the mucociliary function of the stented laryngotracheal region, and also to elucidate the rheological characteristics of laryngotracheal secretions after laryngotracheoplasty with stenting.

## MATERIALS AND METHODS

In our pilot study with a non-radioisotope method, we observed dry resin particles in transit over the laryngotracheal region by an endoscopic technique in tracheostomized guinea pigs. The entire procedure was recorded by videophotography.[2] This method was also used for measuring the resin clearance rate on the stented airway to evaluate laryngotracheal mucociliary transport. In total, 15 healthy Hartley guinea pigs were used. Group 1 was 10 animals in vivo with no stent. Group 2 was 5 animals in vivo, stented.

The body weight of the animals ranged from 300 to 460 g (average 350 g). After overnight fasting for 8 hours, guinea pigs were administered intraperitoneal Nembutal 25 mg/kg (pentobarbital sodium, 50 mg/mL). The animal was maintained at a level of anesthesia with spontaneous respiration but abolished gag or cough reflex.

During the operative procedure, the neck was kept flexed while the head was hyperextended to get a straight airway from the oral cavity to the larynx by placing a small pillow under the animal's shoulder. A vertical anterior midline skin incision was made in the neck. The trachea was exposed by gentle blunt separation to avoid bleeding. Tracheostomy was performed in double stoma fashion about 2.5 to 3 cm distal to the larynx by puncture with an electric cauterization needle. The inferior tracheostoma was used for intubation to control respiration. The upper tracheostoma was used for feeding resin powder that served as a marker substance as well as a material for transport. The resin particles were 10 to 20 μm each and 0.02 mL of powder weighed approximately 7.58 mg. The particles were placed after endoscopy was set up. The upper stoma was sealed. The animals breathed room air spontaneously and the room temperature was kept at 26°C by a closed air ventilation

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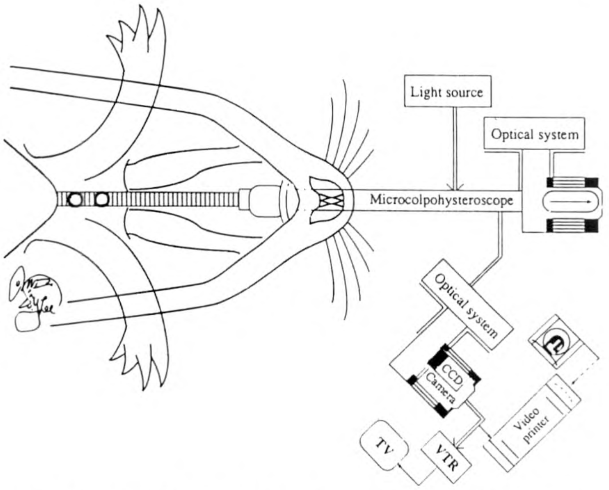

Fig 1. Block diagram of complete experimental preparation of this model. CCD — charge coupled device, TV — television, VTR — videotape recorder.

system. The above surgical procedures were done under the operating microscope.

The peroral endoscopy was performed by introducing a Hamou's microcolpohysteroscope, 4 mm in diameter. The illumination was provided by a 250-W xenon light source. The eyepiece of the scope was adapted to the charge coupled device (CCD, Toshiba) and video camera for video recording. The image from the scope was recorded by a videotape recorder (S-VHS $^1/_2$ in, NTSC; Fig 1). The guinea pig's glottis is suitable in size for clear observation under a 4-mm-diameter microcolpohysteroscope with magnification up to ×60.

For the measurement of mucociliary transit time, the time counting was started as the transport material was fed into the upper tracheostomy fenestra and ended when the particles were viewed on the endoscope at the glottis (Fig 2).

Some procedures for the measurement of the mucociliary transport were carried out after a silicone stent was inserted in the animal's laryngotracheal region with its tip above the cordal level. On animal preparation for the stented group, the endolaryngotracheal stents were inserted gently through the upper tracheal stoma. The size of the stent was the same as that of the ventilating tube, and the distance between the upper tracheostoma and the larynx was the length of the stent, as well as the pathway for the measurement of mucociliary particle transport rate. The upper end of the stent was visualized by the endoscope, which was set up before insertion of marker substance into the upper tracheostomy fenestra. The upper tracheostomy fenestra was sealed after the dry resin powder had been fed. Respiration was kept at a constant rate during the study.

## RESULTS

The results were as shown in Tables 1 and 2. The mucociliary transit time was 6.39 ± 1.27 minutes in the nonstented group and 3.08 ± 0.16 minutes in the stented group. The mucociliary transport rate was 4.41 ± 1.58 mm/min in the nonstented group and 8.62 ± 2.39 mm/min in the stented group.

## DISCUSSION

Many articles have fully elucidated the format of surgical reconstruction of the airway by laryngeal surgeons. Many experts have contributed their methods of stenting: Schuller,[3] Goode and Shinn,[4] Eliachar et al,[5] and Montgomery and Montgomery.[6] Intraluminal stenting with a silicone T-tube has been used for treatment of laryngotracheal stenosis by the authors. We are interested in the mucociliary clearance with and without a stent in place.

Ten animals were used as the control group in

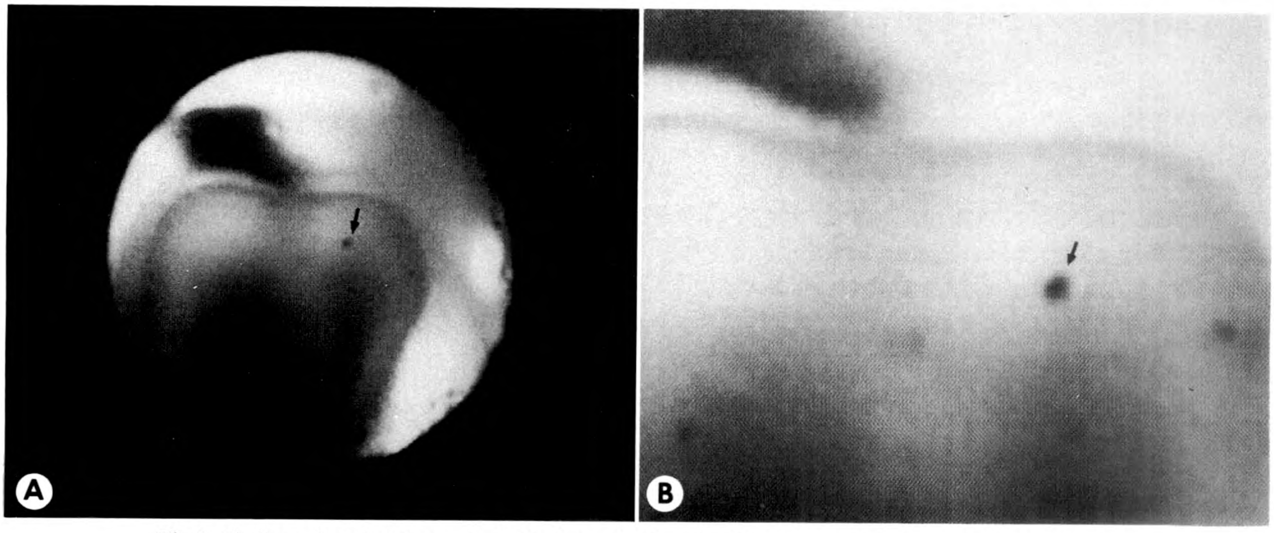

Fig 2. Finding of particle (arrow). A) Endoscopic view. B) Magnification ×60, particle size 15 μm.

TABLE 1. RESULTS OF LARYNGOTRACHEAL MUCOCILIARY TRANSPORT IN GUINEA PIGS

| | *No Stent (n = 10)* | | | *Stented (n = 5)* | | |
|---|---|---|---|---|---|---|
| | *MTT (min)* | *MTR (mm/min)* | *MTL (mm)* | *MTT (min)* | *MTR (mm/min)* | *MTL (mm)* |
| | 7.4 | 4.0 | 30 | 2.9 | 12.4 | 36 |
| | 6.1 | 3.2 | 20 | 3.3 | 6.7 | 22 |
| | 7.0 | 4.3 | 30 | 3.2 | 9.3 | 30 |
| | 7.6 | 3.2 | 24 | 3.0 | 8.0 | 24 |
| | 6.6 | 3.8 | 25 | 3.0 | 6.6 | 20 |
| | 8.3 | 2.7 | 22 | | | |
| | 6.3 | 5.7 | 36 | | | |
| | 5.7 | 5.3 | 30 | | | |
| | 4.3 | 8.1 | 35 | | | |
| | 4.6 | 4.3 | 20 | | | |
| Mean ± SD | 6.39 ± 1.27 | 4.41 ± 1.58 | 27.2 | 3.08 ± 0.16 | 8.62 ± 2.39 | 26.4 |

MTT — mucociliary transit time, MTR — mucociliary transport rate, MTL — mucociliary transport length (distance).

TABLE 2. STATISTICAL ANALYSIS OF LARYNGOTRACHEAL MUCOCILIARY TRANSPORT IN GUINEA PIGS

| | *No Stent (n = 10)* | *Stented (n = 5)* | *Student's t-Test* |
|---|---|---|---|
| Mean MTT (min) | 6.39 ± 1.27 | 3.08 ± 0.16 | p < .005 |
| Mean MTR (mm/min) | 4.41 ± 1.58 | 8.62 ± 2.39 | p < .005 |

MTT — mucociliary transit time, MTR — mucociliary transport rate.

which no stent was placed. Five animals were studied with endolaryngotracheal stenting. The results are shown in Table 2. The mucociliary transit time was 6.39 ± 1.27 minutes in the control group and 3.08 ± 0.16 minutes in the stented group, and the mucociliary transport rate was 4.41 ± 1.58 mm/min in the control group and 8.62 ± 2.39 mm/min in the stented group. All data were treated with Student's *t*-test and had a p value of less than .005, which revealed a significant difference. It is a great challenge to use the guinea pig as an experimental animal for laryngological research in vivo. In the past, it might have been impossible, but recent rapid advances of scientific technique have yielded satisfactory results.

Mucociliary clearance serves as an important respiratory cleansing mechanism in the laryngotracheal region. The detailed transit mechanism is not yet well established at the glottic and subglottic levels. The mechanism involves an interplay of various factors, including inherent mucous properties, laryngeal glottic sphincter action, the swallowing movement, the pressure of the lumen with breathing, and the surface tension of the mucous gel layer. The viscosity and elasticity of the mucus are of great importance for mucociliary transport in a partly damaged area. Clinically, if too extensive an absence of ciliated epithelium renders the mucous transport arrested, pooling of secretions will occur distal to this region. The secretions will soon occlude the lumen. When the stenotic part lacking ciliated epithelium is large enough or when inflammation has paralyzed the cilia, no mucociliary transport is possible. The only way for the patient to get rid of secretions below the affected segment may be insertion of an intraluminal stent to assist the flow of mucus between the stent and the luminal wall. At least some parts of the stenotic area have functional scattered islands of ciliated epithelium that may be enough to maintain satisfactory mucous transport. Thus, luminal stenting is the method of choice either to create a patent airway conduit or to increase the surface tension of the mucus.

During the experiment in nonstented animals, it was revealed that the particles in a mucus globule accumulating at the glottic level and in the interarytenoid region not only relieved glottic sphincteric action but also induced forceful efforts in flow (ie, coughing) that constantly expelled large amounts of mucus with particles. The dry resin particles were moved from the trachea through the glottis, re-collected in the pharynx, then swallowed. In the stented animals, the glottic sphincteric effect was abolished by intraluminal stent insertion, yet there was accumulation in the subglottis that resulted in "coughing." Thus, a longer mucociliary transit time was noted in nonstented animals in vivo. In summary, a stent in the laryngotracheal region may somewhat disturb the sphincteric function grossly. Nevertheless, the mucociliary transport will function as long as there is a smooth surface of airway such as a stent in place. So far, the silicone T-tube is recognized as the best stent for re-epithelization.[1] Indeed, Montgomery and Montgomery[6] have made an invaluable contribution.

## CONCLUSION

In conclusion, the present study demonstrates that an endolaryngotracheal stent may increase the clearance function from a rheological standpoint. It is conceivable that the clinical postoperative status is comparable to the physiologic condition, at least in this preliminary study on an in vivo guinea pig model.

ACKNOWLEDGMENT — The authors thank Professor William W. Montgomery, who read this article before presentation.

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## TWENTIETH ANNUAL MEETING OF THE BÁRÁNY SOCIETY

The Twentieth Annual Meeting of the Bárány Society will be held in Würzburg, Germany, September 20-23, 1998. For more information, contact the Local Organizing Committee, Institute of the 4-G-F, Kurhausstrasse 12, D 97688 Bad Kissingen, Germany; telephone 0049/971-64832; fax 0049/ 971-68637.

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## PEDIATRIC OTORHINOLARYNGOLOGY

The Seventh International Congress of Pediatric Otorhinolaryngology will be held in Helsinki, Finland, June 7-10, 1998. For more information, contact Pekka Karma, MD, Chairman, or Hans Ramsay, MD, Secretary, ENT Department, Helsinki University Central Hospital, Haarmaninkatu 4 E, FIN-00290 Helsinki, Finland; telephone 358 9 4713019; fax 358 9 4715010; E-mail ramsay@hansram.pp.fi.

*Ann Otol Rhinol Laryngol* 106:1997

# POSTTRANSPLANT LYMPHOPROLIFERATIVE DISEASE PRESENTING AS SUDDEN RESPIRATORY ARREST IN A THREE-YEAR-OLD CHILD

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Posttransplant lymphoproliferative disease (PTLD) is an occasional complication of pediatric organ transplantation that, heretofore, has not been associated with airway obstruction. We report the first documented case of PTLD associated with complete airway obstruction resulting in sudden respiratory arrest and death in a 3-year-old child. This is contrasted to a subsequent case of PTLD wherein heightened clinical suspicion and prompt tonsillectomy resulted in a definitive diagnosis and improved outcome. The early clinical hallmarks of PTLD are a mononucleosis-like syndrome, tonsillar enlargement, and positive Epstein-Barr virus seroconversion. The potential for a fatal outcome of PTLD involving Waldeyer's ring components warrants early recognition and aggressive treatment.

KEY WORDS — posttransplant lymphoproliferative disease, respiratory tract.

## INTRODUCTION

Posttransplant lymphoproliferative disease (PTLD) is a well-recognized complication of therapeutic immunosuppression, usually associated with Epstein-Barr virus (EBV) infection.[1] Although EBV is generally considered a self-limited infection in immunocompetent individuals, the virus may be associated with a spectrum of lymphoproliferative diseases in immunosuppressed patients. These entities range from an infectious mononucleosis–like illness to a solid malignant lymphoma.[2]

The presentations of PTLD may vary, depending on the transplant population studied. In a review of 14 pediatric cases of PTLD, Sculerati and Arriaga[3] found that the most common presentation involved hypertrophy of tonsils and adenoids associated with an acute upper respiratory tract illness. Particularly in the pediatric population, seroconversion to EBV may be an early indicator of impending PTLD,[4] as well as new-onset snoring, sleep apnea, and nasal obstruction associated with adenoid and tonsillar hypertrophy.[3] However, PTLD has not been previously reported as causing complete upper airway obstruction with sudden respiratory arrest.

We present a case of sudden respiratory arrest in a pediatric patient with PTLD involving Waldeyer's ring resulting in near-total occlusion of the upper airway. Her history was significant for EBV seroconversion as well as new-onset sleep apnea with snoring. While the clinical literature contains information regarding the association between pediatric organ transplant and involvement of Waldeyer's ring by PTLD, there are no standard guidelines for timing of tonsillectomies and adenoidectomies in these patients. The potential for sudden upper airway obstruction in pediatric PTLD warrants aggressive management despite concurrent upper respiratory tract infection.

## CASE REPORTS

*Case 1.* The first patient was a 3-year-old girl, 26 months status post cardiac transplantation for viral myocarditis and cardiomyopathy. The immunosuppressive regimen included cytotoxic induction with OKT3 and then triple-regimen immunosuppressive therapy (Imuran, cyclosporine, prednisone).

The patient's history was remarkable for seroconversion to EBV 12 months following transplant, as well as seroconversion to *Toxoplasma* and cytomegalovirus (CMV) 18 months posttransplant. Her course was marked by multiple upper respiratory tract infections, for which she was repeatedly admitted to the hospital. None of the routine follow-up endomyocardial biopsies revealed any evidence of infection or

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A portion of this work was presented as a poster at the meeting of the American Society of Pediatric Otolaryngology, Durango, Colorado, May 26-28, 1995.

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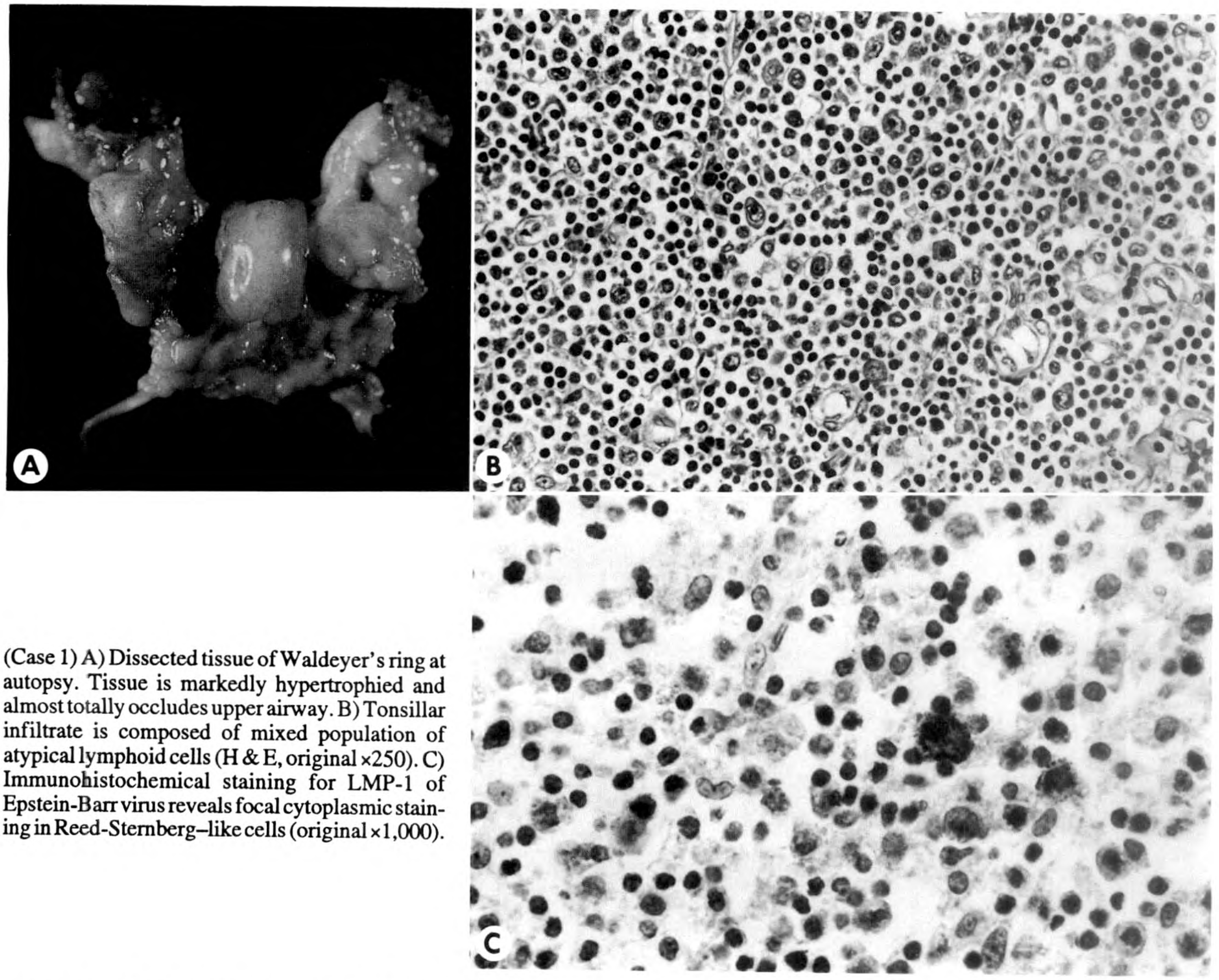

(Case 1) A) Dissected tissue of Waldeyer's ring at autopsy. Tissue is markedly hypertrophied and almost totally occludes upper airway. B) Tonsillar infiltrate is composed of mixed population of atypical lymphoid cells (H & E, original ×250). C) Immunohistochemical staining for LMP-1 of Epstein-Barr virus reveals focal cytoplasmic staining in Reed-Sternberg–like cells (original × 1,000).

allograft rejection. Two weeks before her final hospital admission, she developed enlarged tonsils and adenoids causing sleep apnea. She had been scheduled for tonsillectomy and adenoidectomy at this time; however, the procedure was cancelled because of another acute upper respiratory tract illness.

The day before admission, she was taken to a local hospital with complaints of difficulty breathing and stridor. In the local emergency room, a chest x-ray and electrocardiogram were normal and pulse oximetry showed 97% oxygen saturation. She was discharged home, but the following morning she became cyanotic and collapsed after another episode of stridor. Her mother began mouth-to-mouth resuscitation, and the patient was found to be pulseless by emergency personnel. She was intubated and taken to a local hospital and was transferred later that day.

On admission the child was found to be febrile to 39.4°C, exhibiting decerebrate posturing, twitching of all extremities, and abdominal distention. Three days after admission, her condition deteriorated with hypotension, bradycardia, and hypothermia. Five days after admission she was pronounced dead. An autopsy was performed.

The major autopsy findings involved the lymphoid tissue of the head and neck, lungs, and kidneys. Grossly, the tonsils were tan, fleshy, and hypertrophic, and the surrounding lymphoid tissue (Waldeyer's ring) was also hypertrophied (see Figure, A). The uvula was massively enlarged and edematous and protruded inferiorly to the level of the hyoid bone. The uvula, along with the hypertrophied tonsillar tissue, caused a severe narrowing of the upper airway.

Microscopic study of the tonsils revealed a polymorphous lymphocytic infiltrate of immunoblastic cells, more mature forms, and occasional Reed-Sternberg–type cells (see Figure, B). The atypical lymphoid infiltrate completely effaced the normal tonsillar architecture. The diagnosis of PTLD, polymorphous variant, was rendered. Immunohistochemical staining for UCHL-1 and L-26 (Dako Corporation, Carpinteria, Calif) of this infiltrate revealed a mixed population of approximately equal amounts of B cells and T cells. Similar infiltrates were also present in the uvula, cervical lymph nodes, lungs, and kidneys. No evidence of allograft rejection or graft arteriopathy was seen in sections from the heart. Immunohistochemical staining for latent membrane protein (LMP-1) of EBV was performed on sections from cervical lymph nodes involved by PTLD. Expression of LMP-1 was detected with a commercially available cocktail of monoclonal antibodies, CS 1-4, as the primary reagent (Dako). Numerous cells revealed cytoplasmic staining for LMP-1, including many Reed-Sternberg–type cells (see Figure, C). No evidence for any infection other than EBV was found at autopsy.

*Case 2.* The second patient was a 13-year-old boy, 1 year status post cardiac transplant for dilated cardiomyopathy. His posttransplant course had been marked by chronic cellular rejection despite an immunosuppressive regimen consisting of Imuran, cyclosporine, and prednisone. In February 1994, he was changed to FK 506, and rejection subsequently resolved. However, he developed insulin-dependent diabetes mellitus as a result of the FK 506 regimen. There was no history of opportunistic infections.

He presented to the emergency room with an enlarged erythematous right tonsil with exudate. The otolaryngology consultant recommended tonsillectomy, which was performed 3 days after admission. At that time, EBV IgM and IgG titers were positive and he exhibited a mononucleosis-like illness.

Histopathologic examination of the tonsillectomy specimen revealed PTLD, polymorphous variant, with a mixed infiltrate of immunoblastic cells, more mature forms, and Reed-Sternberg–type cells, as seen in case 1 (see Figure, B). Immunohistochemical staining for EBV was performed on sections from the tonsils, as previously described for case 1. Similarly, focal cytoplasmic staining was seen in many of the atypical lymphoid cells, as well as the Reed-Sternberg–type cells (see Figure, C).

Follow-up clinical studies included computed tomography scan of the neck, chest, abdomen, and pelvis. Several cervical lymph nodes were enlarged, as well as lymph nodes in the pretracheal, precarinal, and retroperitoneal regions. A decrease in the immunosuppressive regimen and treatment with acyclovir was associated with resolution of PTLD. Six months later the patient experienced a recurrence of PTLD in the cervical lymph nodes. Again the immunosuppressive regimen was altered, and treatment with acyclovir was instituted that resulted in resolution of disease.

The patient continued to do well for the next 6 months, until he developed severe occlusive atherosclerosis of the left anterior descending coronary artery. He died awaiting a second cardiac transplant. Postmortem examination revealed total occlusion of the left anterior descending coronary artery and right coronary arteries. No evidence of residual PTLD was found at autopsy.

## DISCUSSION

Posttransplant lymphoproliferative disease is a well-recognized, frequently fatal complication of immunosuppression[5] that encompasses a spectrum of lymphoproliferative disorders that occur following organ transplantation. As described by Hanto et al,[6] three clinical syndromes of PTLD occur in children. The first is a mononucleosis-like syndrome that is usually self-limited. The second, as represented by these cases, begins similarly with a mononucleosis-like syndrome or upper respiratory tract infection, then progresses over the next few weeks or months to widespread lymphoproliferation and death. The third type is an extranodal monoclonal B cell lymphoma that occurs late after primary infection.

There is a strong association between EBV and PTLD.[1-6] Lymphoid tissues are the major target of EBV infection, and cells harboring EBV are observed in organs with evidence of PTLD.[1] Some authors suggest that under immunosuppression a wider range of tissues might become susceptible to EBV.[1] However, as in these cases, expression has not been shown in endomyocardial biopsies posttransplantation.[7] One report showed EBV in cardiac transplant patients in hepatocytes and/or adrenal cortical cells with inflammatory infiltrates, but without the abnormal lymphoid cells characteristic of PTLD.[1] In contrast, case 1, at autopsy, revealed small foci of lymphoid aggregates in brain tissue; however, these areas did not reveal EBV by immunohistochemical techniques.

There has been a moderate to high incidence of lymphoma in organ transplant recipients treated with cyclosporine.[8] Since this association was first described, patients on cyclosporine as part of their immunosuppressive regimen are carefully monitored to maintain an appropriate serum level. Although the frequency of PTLD in adults declined with the introduction of cyclosporine monitoring, there was no decline in PTLD in children.[4] Therefore, pediatric transplant recipients on a cyclosporine regimen must be monitored even more carefully for evidence of PTLD.

The frequency of PTLD in the pediatric transplant population (4%) is much higher than that in the adult transplant population (1%).[4] This difference is thought to be due to the increased rate of primary EBV infection in the pediatric population. Children are at greater risk because they are more likely to be seronegative for EBV prior to transplantation, and to later acquire primary infection.[4] Epstein-Barr virus seroconversion may be an excellent indication for tonsillectomy and adenoidectomy in these patients.

A similar EBV-related lymphoproliferative disorder presenting as tonsillitis has recently been described in adults receiving high-dose chemotherapy in conjunction with total body irradiation following bone marrow transplantation.[9] The 6 patients, described by Yellin et al,[9] all presented with tonsillitis and cervical adenopathy that was refractory to medical treatment. Following surgical intervention, histopathologic diagnosis of non-Hodgkin's lymphoma was rendered for each of these cases. Odynophagia or tonsillitis unresponsive to medical treatment in adult transplant patients may be an indication for tonsillectomy or biopsy to render rapid diagnosis and treatment.

As reported herein, PTLD commonly presents in children as lymphoid hyperplasia or masses in the head and neck region in the setting of an acute febrile illness.[3] In one report of 24 pediatric patients with PTLD, 5 of the 24 cases were first diagnosed in tissue from the tonsils and adenoids.[7] Eight of 12 children in another study presented with new-onset snoring, sleep apnea, or nasal obstruction associated with hypertrophy of tonsils and adenoids.[3] This scenario, especially in the setting of an acute febrile illness, should also suggest surgical intervention.

The outcome of PTLD in children is generally poor.[4] However, in one study in which surgery was aggressively pursued along with reduction in immunosuppression, the mortality rate was 25% during a limited follow-up period.[3] Excision of the obstructing lymphoid tissue is advocated for diagnosis and relief of potential airway obstruction. Systemic therapy is also necessary and includes reduction of immunosuppression. Treatment with acyclovir may be effective in inhibiting the shedding of EBV and has been shown in one study to successfully induce remission in polyclonal B cell proliferations.[2]

Posttransplant lymphoproliferative disease has not been previously reported as causing acute respiratory compromise leading to anoxic brain death. Cases of PTLD should be identified and treated in a timely manner. Clinicians must be aware of the early clinical signs of PTLD in the pediatric population, including hypertrophy of the tonsils and adenoids associated with an acute upper respiratory tract infection. The presentation of the patient must be used as a guideline for appropriate therapy. There must be no delay in excision of potentially obstructing lymphoid tissue, and proper handling of the specimen must follow to allow for an accurate diagnosis. The pathologist should be prepared to receive fresh tonsillectomy and adenoidectomy specimens and to perform immunophenotyping on these tissues. From our experience with the 2 cases presented, as well as data presented in the literature,[3,9] we conclude that early intervention with timely histopathologic diagnosis offers these patients the best chance for good outcome.

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*Ann Otol Rhinol Laryngol* 106:1997

# PREVENTION OF FLASH FIRES DURING FACIAL SURGERY PERFORMED UNDER LOCAL ANESTHESIA

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As more surgical procedures are being performed under local anesthesia and intravenous sedation, complications associated with these techniques are more likely to be experienced. We report a case of an intraoperative flash fire that occurred while supplemental oxygen was being used in this scenario. A literature review and suggestions for prevention of this complication are discussed.

KEY WORDS — ambulatory surgery, burns, fires, intraoperative complications, oxygen.

## PATIENT AND PROBLEM

A 77-year-old woman presented with a 15 × 15-mm basal cell carcinoma of her right cheek (Fig 1). Her past medical history was significant for hypertension, hypercholesterolemia, osteoarthritis, mild chronic obstructive pulmonary disease, and coronary artery disease. Due to the history of cardiopulmonary disease and the anticipated short nature of the procedure, it was decided to perform her surgery with local anesthesia and intravenous sedation.

## OPERATION AND OUTCOME

After arriving in the operative theater, the patient received midazolam maleate, fentanyl citrate, morphine sulfate, and cefazolin sodium intravenously. Thirteen milliliters of 1% lidocaine hydrochloride with 1:100,000 epinephrine were injected subcutaneously in the area of planned surgical excision on the right cheek. Oxygen was administered to the patient at 2 L/min by nasal cannula, and then the skin was prepared with half-strength Betadine solution (10% povidone-iodine diluted 1:1 with sterile water). Sterile cotton towel drapes were placed around the operative site, separating the surgical field from the patient's vision and the oxygen delivery apparatus. A disposable split-sheet paper drape was placed around the cotton towels. The drapes facing the anesthesia machine were suspended such that the patient's face could be directly seen by the anesthesiologist.

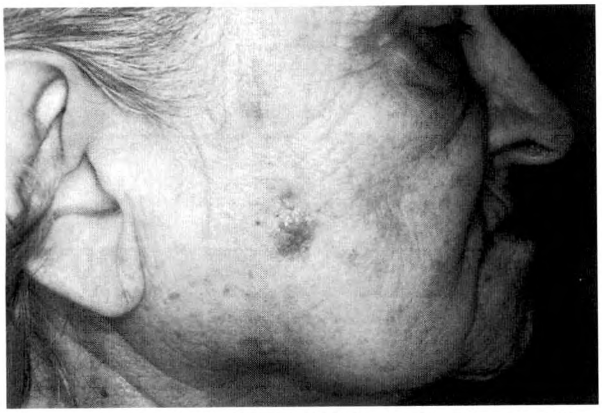

Fig 1. Basal cell carcinoma of right cheek, measuring 15 × 15 mm.

A fusiform wide local excision was performed, axially based along an existing deep cutaneous furrow, and margins were harvested for frozen specimen. A unipolar needle tip electrocautery device, set on 20 W for both cut and coagulation modes, was being used for hemostasis. During use of the electrocautery, a flame appeared that seemed to be originating from beneath the drapes. All drapes and the nasal cannula were immediately removed from the patient, and the flame was noted to be emanating from the nasal cannula. The oxygen delivery was stopped and the flame subsequently abated.

Cool saline-soaked gauze pads were placed on the patient's face immediately. Subsequent inspection revealed sensate facial burns (partial thickness) extending from the patient' right eyebrow diagonally across the nasal area and upper lip to the left perioral area (Fig 2). There was minimal burn to the inner nasal vestibule and the oral cavity was unharmed. The patient was in no respiratory distress but did complain of pain from the burns. A Wood's lamp fluorescein dye test, done in the operating room, revealed no corneal or scleral injury, and a subsequent postoperative ophthalmologic consult confirmed no injury to the globe.

Examination of the drapes and oxygen delivery

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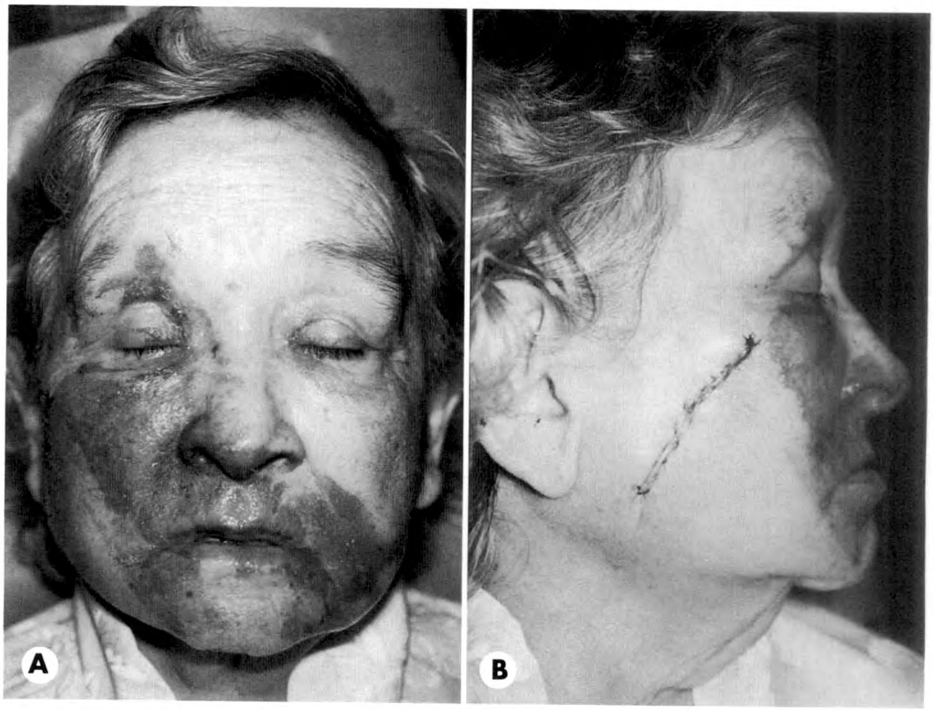

Fig 2. Photographs taken 24 hours postoperatively, showing well-demarcated extent of partial thickness burns. A) Frontal. B) Lateral.

system revealed a 3 × 3-cm charred area of one of the blue cotton towel drapes, and the distal portion of the nasal cannula had been melted (Fig 3). The split-sheet drape had no evidence of burns.

The patient's burned areas were kept covered with saline-soaked gauze pads for the remainder of the case. A new oxygen nasal cannula was placed on the patient at 2-L/min delivery, and her face was not draped for the remainder of the procedure. Further hemostasis was achieved with silver nitrate cautery sticks and local pressure. The wound was closed following the returned report of negative surgical margins by frozen section. Neomycin sulfate ointment was applied to the surgical wound and burned areas, with ophthalmic neomycin sulfate applied to the right periorbital area. The patient was returned to the recovery room without further event, and has subsequently healed from her burns without scarring or permanent sequelae.

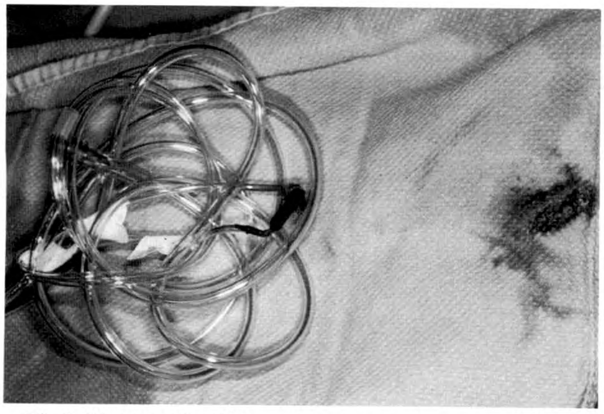

Fig 3. Photograph of burned nasal cannula oxygen delivery device and charred cotton towel removed from patient.

## DISCUSSION

The focus in dermatologic surgery has shifted toward ambulatory settings over the past decade. In-office procedures continue to become more routine, with the vast majority of these procedures being done under sedation and local infiltration of anesthesia. Supplemental oxygen delivery is utilized by many surgeons and anesthesiologists in the United States, with monitoring of patient oxygenation via pulse oximetry. The use of a nasal cannula is commonplace in this setting. The experience of an intraoperative flash fire has caused us to reconsider the routine use of supplemental oxygen.

Three requirements exist for combustion to occur: fuel, an oxidizing source, and a heat source. Fuel sources that have been described in the literature as possible contributors to intraoperative fires include drapes, gauze sponges, patient tissues, oxygen delivery devices, alcohol-based scrubs (not commonly used in the United States), flammable hair sprays or perfumes worn by the patient, aerosolized tincture of benzoin, Weck-cel sponges, intravenous tubing, esophageal stethoscopes, and flammable organic fumes produced by necrotic epithelial tumors.[1-8] Some suture materials are stored in flammable preservative fluids, and at least one case of a resulting intraoperative fire has been reported.[9] Catgut and some nylon sutures are stored in solutions containing up to 90% isopropyl alcohol. The individual suture packets do not detail this information; the box does list the preservative content, but without a fire hazard warning. Both oxygen and nitrous oxide are powerful oxidizing agents, and the combination may support combustion better than 100% oxygen.[10-12] Heat sources may include electrocautery devices, lasers, faulty electrical equipment, and drilling and burring devices.

In our patient, it seems most likely that a spark (although not actually seen by any of the personnel involved in the case) from the electrocautery device ignited the patient's tissues, with the process being then sustained by the oxygen flowing through the cannula. The cannula (a polyvinyl chloride product) and cotton drape would than have been secondarily ignited. No flames were ever actually seen on the drapes during the brief fire. It is certainly possible that the drapes or nasal cannula may have been the initially ignited item, however, and these possibilities have not been excluded.

Both disposable and cotton surgical drapes are commonly used in the operative setting. The fire potential for either seems obvious. The use of moistened cotton towels appears a viable option with this in mind. Disposable surgical drapes, most of which are cellulose-based, have primarily evolved from the need to decrease medical costs and to decrease the theoretic potential for spread of infectious disease. They are also more water-repellent and less permeable than cloth drapes.[13] However, they also burn more readily.[14] Even if the drapes are treated with flame-retardant chemicals, fires may spread with amazing speed when they are ignited, and significant fires have occurred with resulting patient deaths.[8,13-15] Because these drapes are water-repellent, dousing the flames with water is not effective.[8] Adhesive drapes may be more difficult to remove promptly in an emergency. A report of ignition of a space blanket, used to maintain a patient's body temperature, also exists.[16] In addition, nasal cannulas are made of combustible plastic,[16] as was noted in our case. Polyvinyl chloride tubing produces an intense flame when ignited, possibly due to the release of vinyl chloride gas, which is highly flammable.[17]

The existence of an oxygen-enriched environment facilitates the ignition and combustion of any material.[18] The Apollo spacecraft fire in 1967 is a notorious example of this phenomenon. Placing drapes over an oxygen delivery source allows potential for pockets of oxygen-enriched air to develop.

A study by Greco et al[19] showed that progressively increasing oxygen concentrations occur beneath operative drapes while the levels of oxygen delivery are increased. Levels as high as 53.5% were obtained with an oxygen flow rate of 6 L/min. Oxygen levels in the open, undraped area of their operative field never rose above 26.3% at 6 L/min, an elevation that did not reach statistical significance. They also measured oxygen levels in an open-face (undraped) model and found no increased concentration with up to 6 L/min delivered. In another arm of their study using delivery of compressed room air, they found that although oxygen concentrations beneath the drapes decreased to as low as 14.9%, the patient's oxygen saturation never decreased below 96% at 5 L/min.

Another study evaluated the minimal conditions needed for combustion to occur with the use of the electrocautery and supplemental oxygen delivery during the open-face technique.[20] The investigators were unable to cause a flash fire, despite using 5 L/min oxygen and an approximately 50-W coagulation setting on the electrocautery, which was used as close as 1 cm from the nasal cannula oxygen source, when the nasal cannula was properly placed. This study nicely complements that by Greco et al,[19] showing the safer environment of the open-face technique for facial surgery when oxygen supplementation is in use.

## LESSON

We have adopted a new approach to performing ambulatory facial surgery under local anesthesia. We now limit supplemental oxygen to those patients who truly need it, as dictated by their concurrent health conditions. On occasion, oxygen will temporarily be needed due to the respiratory depression caused by intravenous sedation with resulting oxygen desaturation. When supplementation is warranted, we use the lowest inspired oxygen concentration necessary to allow acceptable oxygen saturation levels. We also use an open-face technique whenever possible, and ensure that the nasal cannula is properly placed. All patients are continuously monitored throughout the procedure with pulse oximetry. As the well-known oxygen-hemoglobin dissociation curve demonstrates, maintaining saturation levels above 90% is paramount for maintaining an edge of safety in healthy patients. Higher oxygen levels are warranted in patients with additional compromising medical conditions. This population will likely need supplemental oxygen throughout the case. While we do not attempt to claim that oxygen supplementation should never be routinely delivered during all such cases and acknowledge differing opinions, at our institution we have adopted a different approach. We are using a protocol in which oxygen supplementation is not considered routine for all cases, but is delivered with the same rationale and caution one should have for the use of any pharmacologic agent. We have had no untoward sequelae to date using this approach.

We have found a small population of patients who prefer not to be able to see the operative site during the procedure. For these patients, we are draping out only the eyes with moist cotton towels. If oxygen supplementation is medically indicated, the nasal cannula is not included beneath the drapes. Oxygen saturation levels are continuously monitored with pulse oximetry, and oxygen supplementation is given only as needed, and for only as long as needed.

## SUMMARY

In performing facial surgery under local anesthesia with intravenous sedation, consideration should be given to preventing flash fire misadventures. Although they are seemingly rare occurrences, the results can be catastrophic. We report a case of an operative flash fire that occurred during the use of low-setting electrocautery on a patient receiving 2 L/min oxygen by properly placed nasal cannula, with the operative field being draped away from the oxygen source. While our patient sustained only minimal injury, the potential for more severe consequences is acknowledged. This has changed our current management of these patients. We have adopted the use of an open-face technique, using oxygen supplementation via nasal cannula only either when warranted by a patient's preexisting health problems or when decreased ventilation and oxygenation occur as a result of the respiratory depressive effects of sedation. When draping becomes necessary by patient preference, we drape out only the eyes, using moist towels. We use continuous pulse oximetry in all cases.

Perhaps most important is to reflexively know what to do should a flash fire occur. All drapes, sponges, and other flammable materials should be immediately removed from the patient. Likewise, any oxygen delivery devices should be immediately removed and the anesthesiologist asked to stop the oxygen flow. Cool, moist sponges should be applied to the wounds, and only then should the extent and depth of injury be assessed.

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## IMAGING CASE STUDY OF THE MONTH

# BROWN TUMOR IN THE CRICOID CARTILAGE: AN UNUSUAL MANIFESTATION OF PRIMARY HYPERPARATHYROIDISM

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## INTRODUCTION

Primary hyperparathyroidism (HPT) is accompanied by a vast array of symptoms and the occurrence of brown tumors that arise predominantly in the metacarpals, phalanges, jaws, pelvis, and long bones.[1] The following is the first case report known to us of a brown tumor in the laryngeal cartilage.

## CASE REPORT

An 84-year-old, usually healthy woman broke her right ankle. Radiography of the ankle showed the fracture and severe osteoporosis. Because of diffuse bone pain, a bone scan was performed and multiple hot spots were demonstrated in both legs, the right greater trochanter, the right sternoclavicular joint, the right scapula, and the ribs. Radiographs of these areas showed multiple osteolytic lesions, with marginal fractures in some of them. The patient was referred to the oncology service for further evaluation. On physical examination the patient presented with mild to moderate shortness of breath and hoarseness with accompanying weakness and tenderness of the right arm and forearm.

Findings on laboratory tests including complete blood cell count, erythrocyte sedimentation rate, electrolytes, urea, creatinine, liver transaminases, and serum protein were normal. The calcium level was 10.2 mg% (2.5 mmol), phosphorus 2.7 mg% (0.87 mmol), and alkaline phosphatase 666 IU.

Fine needle aspiration biopsy from the lesion in the right scapula revealed brown material rich in multinucleated giant cells compatible with brown tumor. Repeat calcium and phosphorus levels were 10.7 mg% (2.7 mmol) and 2.5 mg% (0.87 mmol), respectively. The parathormone level was 221 pg/mL (normal 10 to 60), confirming the diagnosis of primary HPT. Localization studies for the adenoma were performed. Ultrasound scanning of the neck showed a right parathyroid mass, 20 mm in diameter.

Computed tomography (CT) of the neck demonstrated a 10 × 15-mm right-sided retrothyroid nodule suggesting a parathyroid adenoma (see Figure, A). Slightly more caudal slices demonstrated a well-defined, expansile 14 × 24-mm lesion destroying the left half of the cricoid cartilage. The mass enhanced homogeneously after intravenous contrast injection and caused narrowing and deformity of the adjacent trachea (see Figure, B). Direct laryngoscopy revealed a smooth, pink left subglottic mass beneath and abutting the left vocal cord. Biopsies were inconclusive. A CT-guided fine needle aspiration of the cricoid mass revealed only blood and was nondiagnostic.

Open biopsy of the mass produced bone tissue with multinucleated giant cells, compatible with brown tumor of HPT. The patient underwent neck exploration and a right parathyroid adenoma (20 × 15 mm) was removed. The postoperative period was uneventful, except for a few days of hypocalcemia that was treated by oral calcium. Follow-up CT examination of the larynx revealed no change in the cricoid lesion.

## DISCUSSION

Brown tumors (osteitis fibrosa cystica) in HPT are caused by localized, rapid osteoclastic turnover of bone, secondary to a direct effect of parathormone on bone. As a result, hemorrhage, reparative granulation tissue, and active, vascular, proliferating fibrous tissue replace the normal marrow contents.[2]

The term brown tumor is derived from the characteristic macroscopic appearance of brownish material within this cystic lesion, secondary to blood pigments that are both free and within hemosiderin-

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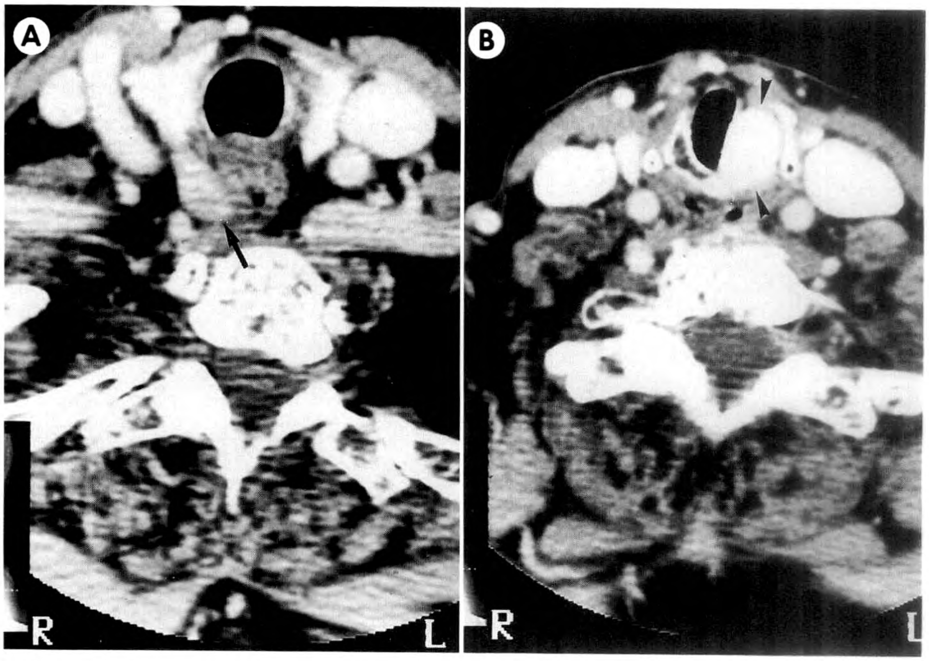

Contrast-enhanced computed tomograms. A) At level of thyroid gland, demonstrating enhancing mass posterior to right thyroid lobe (arrow). B) At cricoid level, showing enhancing, expansile, well-defined lesion occupying left aspect of cricoid cartilage, causing narrowing and deformity of trachea (arrowheads).

laden macrophages. These "tumors" are hypervascular and intensely active on bone scans.[3] Although brown tumor can arise in the jaws and involve the paranasal sinuses,[4] they are very unusual in daily otolaryngologic practice.

A computed tomography scan is an excellent noninvasive procedure to ascertain the topography of the site and to delineate the extent of the pathologic lesion.

The CT appearance of brown tumor is of an expansile, usually well-defined lesion. A CT-guided needle aspiration biopsy can provide cytodiagnosis of osteitis fibrosa cystica showing hemorrhage, multinucleated giant cells, and fibrous tissue.[5] In our case the CT-guided fine needle aspiration biopsy revealed only blood and was inconclusive. Repeated biopsy under direct vision was diagnostic for brown tumor.

Brown tumors are radiologically and histologically hard to distinguish from giant cell tumors.[6] Nevertheless, expansile, well-defined lytic lesions, especially when numerous, in a patient with known HPT are more likely to be brown tumors than giant cell tumors.

The cricoid cartilage is composed of hyaline cartilage, but it commonly becomes calcified later in life. Ossification may also occur, and therefore bone tumors such as osteosarcomas, as well as chondroid matrix tumors such as chondromas and chondrosarcomas, may arise in the cricoid cartilage.[7] This can also explain the presence of a brown tumor, which is basically an osseous process in this unusual location.

Essentially, brown tumors are benign, but emergency surgery for tumor removal is indicated when the lesion is located in a problematic site. Our patient was not operated on, because the symptoms of hoarseness and difficulty in breathing were subtle.

In conclusion, this is the first reported case of a brown tumor in the cricoid cartilage. An accurate diagnosis can be made by considering brown tumor in the differential diagnosis of a laryngeal cartilage lesion and taking into account clinical and laboratory data as well as accompanying skeletal findings.

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## CLINICOPATHOLOGICAL CONSULTATION

# YOLK SAC TUMORS (ENDODERMAL SINUS TUMORS) OF THE EXTRACRANIAL HEAD AND NECK REGIONS

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Yolk sac tumors (or endodermal sinus tumors) are rare neoplasms of germ cell origin that have been reported in gonadal sites (testis and ovary). Extragonadal yolk sac tumors are uncommonly seen, and in particular, they are rare in the extracranial head and neck regions; we found 27 well-documented cases in the English-language literature. We discuss their clinical and pathologic features.

## INTRODUCTION

Germ cell tumors are lesions composed of tissues that appear to have been derived (in many cases) from each of the three basic embryonic tissue types — that is, from the ectoderm, the mesoderm, and the endoderm. Germ cell tumors may be benign or malignant, and may be diagnosed in newborns, older children, and adults.[1,2] Germ cell tumors have been diagnosed in a variety of sites, including the ovary, the testis, the mediastinum, the retroperitoneum, and the sacrococcygeal region. They are rarely seen in the extracranial head and neck regions; when germ cell tumors are encountered by the otolaryngologist, they are seen most frequently in patients in the pediatric age group.[3]

As noted above, germ cell tumors may be benign or malignant; in the extracranial head and neck regions in particular, germ cell tumors are most often benign (teratomas).[3] Among the recognized histologic subtypes of malignant germ cell tumors, the yolk sac tumor (YST) is the second most common type of malignant germ cell tumor encountered (following behind the seminoma, or dysgerminoma); over three quarters of the YSTs that have been reported have arisen in the ovary and the testis.[1,4,5] It is, therefore, rather unusual for surgeons to encounter this tumor type in an extragonadal location such as the extracranial head and neck regions.[4,6] As a consequence of the rarity with which these tumors are found in the extracranial head and neck territory, they may pose problems for both the treating surgeon and the diagnostic pathologist (and in fact, in the past, are likely to have been underdiagnosed); as a consequence, this report will detail some of the clinical and pathologic features of head and neck YST in an attempt at bolstering the ability of physicians to diagnose and treat these uncommon tumors.

## CLINICAL FEATURES

There are at least 27 well-documented cases of YST of the head and neck region (exclusive of the central nervous system) in the English-language literature; the clinicopathologic features of these cases are summarized in Table 1.[4,7-20] Additional cases of YST are referred to in larger series of germ cell tumors of the head and neck region.[6] Several points may be made from the data collected in Table 1; to begin, a minority of the cases of YST developed in children who had been previously found to have mature teratomas in the same vicinity. It is possible that minor components of YST were present within the bulk of the initially resected "benign" teratomas — which serves to point up the importance of a thorough sampling of such putative benign lesions for histologic study so as to exclude the possibility that an occult malignant component may be lurking among the benign elements. A second observation relates to the age and sex distribution: the vast majority of cases of head and neck YST arise in children and exhibit a female predominance. Neither of the two adult patients with YST in this region have had pure tumors. Rather, both of these lesions were associated with a nonkeratinizing sinonasal squamous carcinoma (transitional cell, or cylindric cell, carcinoma) component. This raises the possibility that the tumors may develop differently among members of the two respective age groups. That is, the pediatric tumors may actually derive from germ cells while the adult lesions may develop by the process of "dedifferentiation" or (less felicitously) by "metaplasia" within an epithelial malignancy that had previously developed in the sinonasal tract.

The typical presenting signs in patients with YST relate to the presence of a rather rapidly growing mass

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TABLE 1. CLINICAL FEATURES OF 27 PATIENTS WITH HEAD AND NECK YOLK SAC TUMORS

| *Case No.* | *Ref.* | *Age* | *Sex* | *Location* | *Other Associated Tumor* | *Treatment* | *Follow-up** |
|---|---|---|---|---|---|---|---|
| 1 | 9 | Newborn | M | Anterior neck | Mature and immature teratoma | Surgical excision | Alive, NED 13 y |
| 2 | 9 | 6 wk | F | Forehead | Undifferentiated carcinoma | Incisional biopsy, chemotherapy, radiotherapy | DOD $4^1/2$ mo |
| 3 | 12 | 3 mo | N/A | Orbit | None | N/A | N/A |
| 4 | 9 | $4^1/2$ mo | F | Facial region | Mature and immature teratoma | Surgical excision | Alive, NED $10^1/2$ mo |
| 5 | 4 | 6 mo | F | Floor of mouth | None | Partial excision, radiotherapy | DOD 7 mo |
| 6 | 18 | 10 mo (tumor first noted at 2 mo by history) | F | Maxillary sinus | None | Excisional biopsy | DOD 1 mo |
| 7 | 4 | 10 mo | F | Nasopharynx | None | Incisional biopsy, chemotherapy, radiotherapy | DOD 9 mo |
| 8 | 4 | 11 mo | F | Oropharynx | Mature teratoma (excised at 20 days of age) | Surgical excision, chemotherapy, radiotherapy | DOD 4 mo |
| 9 | 12 | 1 y 1 mo | F | Orbit | None | Incisional biopsy, steroids, chemotherapy | DOD 10 mo |
| 10 | 12 | 1 y 1 mo | M | Orbit | None | Exenteration | Alive, NED 8 y |
| 11 | 10 | 1 y 3 mo | M | Orbit | None | Incisional biopsy, chemotherapy, radiotherapy, eventual enucleation | Alive, NED 2 y |
| 12 | 12 | 1 y 6 mo | M | Orbit | None | Incisional biopsy, chemotherapy, radiotherapy | Alive, NED 8 y |
| 13 | 20 | 1 y 7 mo | F | Submandibular region | None | Incisional biopsy, chemotherapy, radiotherapy, surgical excision | DOD 5 mo |
| 14 | 14 | 1 y 7 mo | F | Ear | None | Incisional biopsy, chemotherapy | Alive, NED 15 mo |
| 15 | 19 | 1 y 9 mo | F | Temporal region | None | Surgical excision, chemotherapy | Alive, NED 16 mo |
| 16 | 7 | 1 y 10 mo | F | Orbit | None | Surgical excision, chemotherapy | N/A |
| 17 | 15 | 1 y 11 mo | F | Temporomandibular region | None | Incisional biopsy, chemotherapy | Alive, NED 40 mo |
| 18 | 16 | 2 y | M | Nasopharynx | None | Surgical excision, chemotherapy, radiotherapy | Alive, NED 4 y |
| 19 | 17 | 2 y | F | Parotid gland | None | Surgical excision, chemotherapy, radiotherapy | DOD 18 mo |
| 20 | 9 | 2 y 6 mo | F | Retroauricular region | Undifferentiated anaplastic carcinoma | Partial excision, chemotherapy, radiotherapy | Alive, NED 3 y |
| 21 | 8 | 3 y | F | Nasopharynx and paranasal sinuses | Mature teratoma (excised in neonatal period) | Surgical excision, chemotherapy, radiotherapy | DOD 18 mo |
| 22 | 15 | 3 y 8 mo | M | Neck region | None | Incisional biopsy, chemotherapy, radiotherapy | DOD interval unknown |
| 23 | 13 | 4 y | M | Cheek region | None | Incisional biopsy, chemotherapy, radiotherapy | Alive, NED $5^1/2$ y |
| 24 | 12 | 4 y | F | Orbit | None | Exenteration, chemotherapy, radiotherapy | Alive, NED 10 y |
| 25 | 13 | 6 y | F | Mandibular region | Mature teratoma (excised at 10 mo of age) | Surgical excision, chemotherapy, radiotherapy | DOD 18 mo |
| 26 | 11 | 34 y | M | Nasopharynx | Sinonasal squamous carcinoma (nonkeratinizing subtype) | Partial surgical excision, chemotherapy, radiotherapy | DOD 12 mo |
| 27 | 11 | 43 y | M | Nasopharynx and paranasal sinuses | Sinonasal squamous carcinoma (nonkeratinizing subtype) | Partial surgical excision, chemotherapy, radiotherapy | AWD and lung metastases 6 mo |

Ref. — reference, N/A — not available, NED — no evidence of disease, DOD — dead of disease, AWD — alive with persistent disease.

*Times given are post-diagnosis.

lesion; the mass may be painless or painful, and may be accompanied by systemic signs such as fever.[7,17] The mass, as a function of its location, may produce complaints ranging from proptosis to dyspnea to a cosmetic deformity.[7,10,16,18,19]

Imaging studies will reveal a homogeneous soft tissue density on both plain film radiographs and computed tomography (CT) scanning; the lesion may also be homogeneous on ultrasound and exhibit increased uptake on technetium scanning.[6,7,10,14,15,17] Bony erosion may be appreciated, particularly on CT scanning.[6,14] Calcification within the mass (as detected by plain films or CT scanning) has not been noted in the majority of cases. (One report makes reference to a history of juxta-osseous calcification within one lesion, but this may well have represented periosteal new bone formation in the vicinity of the mass lesion.) Nevertheless, calcifications are sometimes encountered in teratomas, and so it is conceivable that a YST that develops in association with a teratoma may, on imaging studies, show foci of calcification. Experience with magnetic resonance scanning is limited to a single case; in that case, the YST was relatively dark on T1-weighted images and relatively bright on T2-weighted images.[7]

It might also be mentioned at this point that in view of the greater incidence of YST in gonadal than in extragonadal sites (such as the head and neck), a tentative diagnosis of a primary YST in the province of the otorhinolaryngologist should prompt that treating physician to exclude on clinical grounds the possibility that the lesion may actually represent a metastasis from another site.

## PATHOLOGIC FEATURES

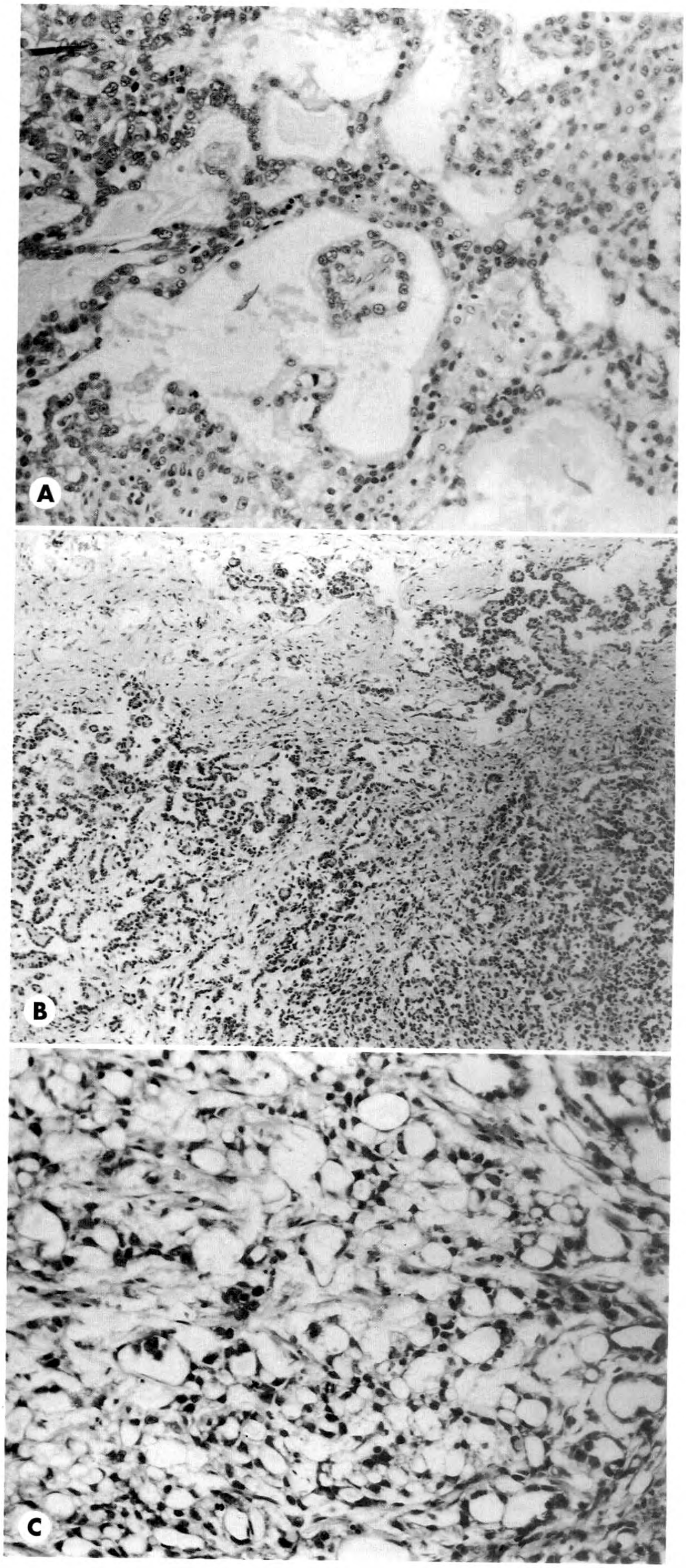
In the head and neck region, the YST may appear as a component of a teratoma (that is, the YST may accompany benign tissues of multiple germ line origins, such as epithelial and cartilaginous tissues, which are not native to the area and thus would by themselves be diagnosed as teratomatous), and so the lesion may be diagnosed either as a malignant teratoma with specification of the presence of a YST component, or as a YST with accompanying benign teratomatous elements. Conversely, a lesion may consist of pure YST, a situation in which the tumor may be approached as a "monophasic" malignant teratoma (malignant germ cell tumor) — that is, a tumor in which the expected benign teratomatous elements are simply not identified (and it is this situation that appears to be the most frequent in YSTs arising in the head and neck region). Finally, it is possible that YST elements may not be the sole malignant component of a given lesion, and may be accompanied by non–germ cell malignancies (such as adenocarcinoma or chondrosarcoma) or even, for that matter, by other malignant germ cell tumor types (as, for example, embryonal carcinoma).[9]

The basic cell type that comprises the YST is a polygonal or flattened cell with a relatively hyperchromatic vesicular nucleus, distinct small central to paracentral nucleoli, and clear to eosinophilic cytoplasm; spindling of the tumor cells may occur as well, particularly in the setting of a myxoid stroma.[21] A moderate to pronounced degree of pleomorphism is encountered among the tumor cells. Mitotic figures, while not present in overwhelming numbers, are usually readily identified in most high-power fields within the tumor.

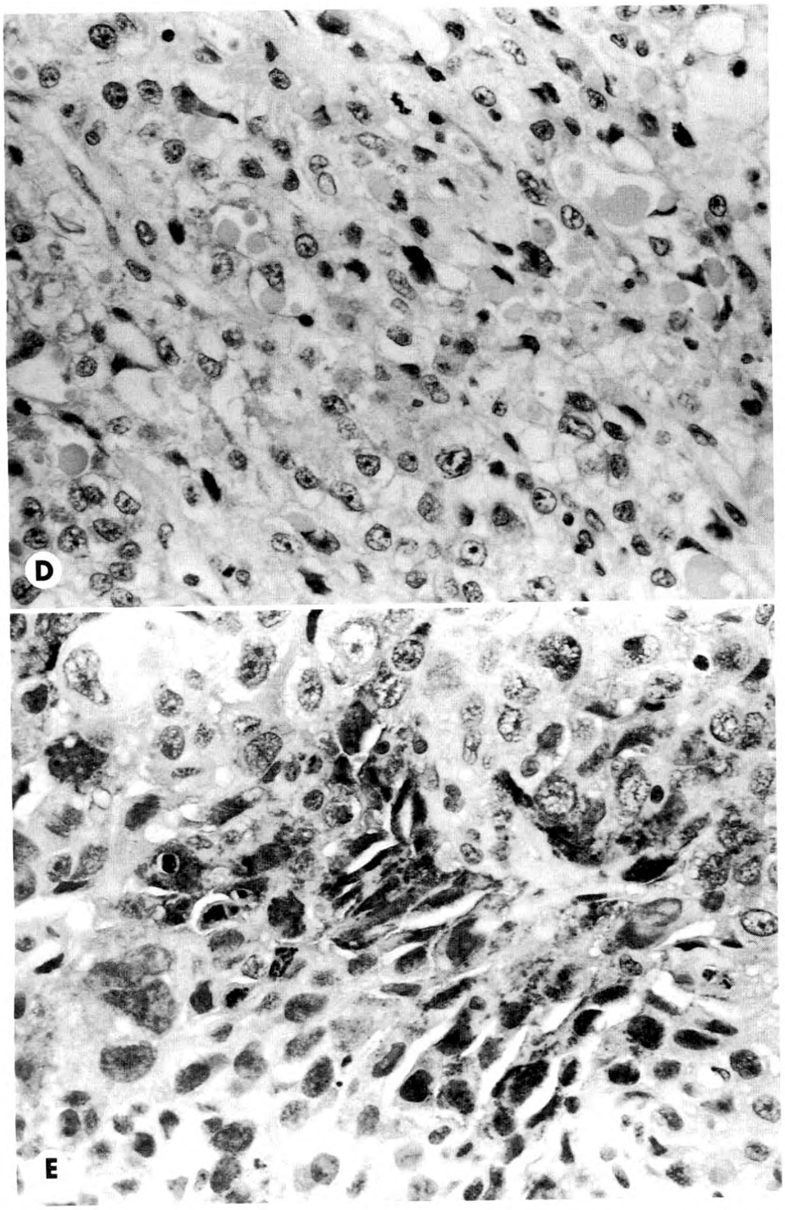
With regard to architecture, the tumor cells of a YST may be arranged in a bewildering variety of patterns; it is usually the case that more than one of these patterns will be found (on thorough sampling) in a single patient's lesion. In view of the rarity of these tumors in the head and neck area, the full list of potential architectural types that may be encountered will be inferred from the (much more frequently encountered) ovarian lesion; these various patterns number at least 10, and include 1) perivascular (endodermal sinus Schiller-Duval bodies), 2) microcystic, 3) macrocystic, 4) solid, 5) alveolar and/or glandular, 6) polyvesicular vitelline, 7) myxomatous, 8) papillary, 9) hepatoid, and 10) intestinal (primitive endodermal) patterns.[22] While it has been suggested that the histologic pattern adopted by a given YST arising in the ovary may show some relation to the course of disease, it is not clear what bearing such an observation may have on the treatment of patients with head and neck YSTs.[23,24]

In general, the architectural patterns most often adopted by YSTs include arrays of a single row of tumor cells about a central blood vessel, which are in turn surrounded by a cystic space (the structures known as Schiller-Duval bodies; the finding of these structures initially suggested to Gunnar Teilum[5] a resemblance of this tumor type to the normal histology of the rat placenta, and hence the name of the human tumor as YST; see Figure, A), a tubulopapillary arrangement (see Figure, B), a vacuolated microcystic pattern in which the tumor cells adopt a spindled configuration in some areas (see Figure, C), and a solid (sheetlike) arrangement of polygonal tumor cells (see Figure, D). These patterns often appear in combination with one another in a given tumor.

Eosinophilic globules that usually (but not invariably) prove, on histochemical study, to be periodic acid–Schiff–positive and diastase-resistant may be



Yolk sac tumor. A) Of orbit, with cystic spaces lined by cuboidal tumor cells as well as perivascular rows of tumor cells that are in turn located within cystic spaces (Schiller-Duval bodies; center, upper right; H & E, original ×200). B) Nasopharyngeal, in which cuboidal tumor cells are disposed in predominantly tubulopapillary arrangement (H & E, original ×250). C) Of orbit, with vacuolated microcystic appearance; here, tumor cells vary in shape from cuboidal to flattened to spindled (H & E, original ×200). (Continued on next page.)



(Continued) D) Of orbit, in which tumor cells form solid unrelieved sheet; there are, in addition, several scattered intracytoplasmic and extracellular globules present here that will prove, on histochemical staining, to be periodic acid–Schiff–positive and diastase-resistant (H & E, original ×340). E) Of orbit, in which majority of tumor cells exhibit finely granular cytoplasmic positivity for α-fetoprotein (immunoperoxidase, antibody to α-fetoprotein, original ×420).

found in both the cytoplasm and the extracellular space in over three quarters of YSTs (see Figure, D).

On immunohistochemical study, the eosinophilic globules noted on light microscopy are usually negative with antibody to α-fetoprotein; however, the cytoplasm of the tumor cells is almost always strongly positive for α-fetoprotein (see Figure, E).[25] The finding of α-fetoprotein positivity does not, however, in itself prove a diagnosis of YST, as embryonal carcinomas and hepatocellular carcinomas, among other lesions, may exhibit strong α-fetoprotein positivity as well. The cells of YST are also consistently positive immunohistochemically for cytokeratin. Clearly, the pathologist is best advised to combine the light microscopic, immunohistochemical, and clinical features of a lesion in a given patient so as to reach a confident final diagnosis of YST in the head and neck territory.

While ultrastructural features of the YST are not, in themselves, diagnostic of the entity, these features have been explored and may in some instances provide some support for a tentative diagnosis of YST. The tumor cells are marked by readily identifiable, well-formed desmosomal junctions, as well as by a most characteristic stromal finding: an abundance of (discontinuous) basement membrane material.[26,27] The tumor cell nuclei are irregular, with dispersed chromatin; multiple nucleoli may be present. Cytoplasmic components include plentiful glycogen deposits (which may be aggregated), rough endoplasmic reticulum, mitochondria, and scattered lipid vacuoles. True glandular lumina lined by relatively short, delicate microvilli may be found. Large, rounded, electron-dense aggregates of amorphous material

TABLE 2. CHARACTERISTIC FEATURES OF HEAD AND NECK YOLK SAC TUMORS

- Clinical features
  - Typically, mass lesion
  - May be painful or painless
  - Orbit is particularly favored head and neck site, although may develop anywhere in head and neck territory
  - Soft tissue density on plain films, computed tomography, and magnetic resonance imaging—possibly with destruction of adjacent bone — usually not calcified
- Light microscopy
  - Epithelial cell lesion in which tumor cells may be arranged in variety of potential architectural arrangements, including perivascular (endodermal sinus, or Schiller-Duval pattern), microcystic, macrocystic, solid, alveolar (glandular), polyvesicular vitelline, myxoid, papillary, hepatoid, and intestinal (primitive endodermal) patterns
- Supporting studies
  - Markedly elevated serum α-fetoprotein levels
  - Positive immunohistochemical staining of tumor cells with antibody to α-fetoprotein
  - Frequent chromosomal abnormalities, affecting in particular chromosomes 1, 3, and 6

appear both within the cytoplasm of tumor cells and in the extracellular space; the intracellular globular aggregates are typically membrane-bound, while the extracellular aggregates are not. These electron-dense structures correspond to the periodic acid–Schiff–positive and diastase-resistant structures noted on light microscopy. Microcyst formation (identifiable, in at least some areas, as dilated endoplasmic reticulum) is often a well-developed feature within the tumor cells. The tumor stroma is rather undistinguished, with scattered electron-dense particles and random collagen fibers.

## SPECIAL SUPPORTING STUDIES

Serum levels of α-fetoprotein will, in the majority of instances, be significantly elevated — both in patients with localized disease and in those in whom metastatic deposits have developed.[28] Should the possibility of another component of malignant germ cell tumor (malignant teratoma) be considered, then serum determination of β–human chorionic gonadotropin (elevated in choriocarcinoma) may be of some aid in selected cases; when considering the possibility of a metastasis from another solid organ (such as gastrointestinal tract, lung, or breast), a sizeable elevation in serum carcinoembryonic antigen may be found. In general, neither β–human chorionic gonadotropin nor carcinoembryonic antigen is greatly elevated in the typical patient with a pure YST. As was the case with immunohistochemical staining of tumor cells with antibody to α-fetoprotein, an elevated serum α-fetoprotein is not in itself diagnostic of YST, as such an abnormality may accompany other tumors (including hepatocellular carcinoma and pancreatic carcinoma), as well as nonneoplastic conditions such as viral hepatitis. It should also be borne in mind that a YST may actually represent only a single component of a complex lesion (theoretically, that is, it is possible that a head and neck YST may accompany, for example, a choriocarcinomatous component).

Cytogenetic studies of YST in children from a variety of sites have shown an association of this tumor type with subtle abnormalities of the short arms of chromosomes 1 and 3 and the long arm of chromosome 6; it remains to be seen what bearing such findings may have on diagnosis or prognosis, however.[29]

The key diagnostic features of YST arising in the head and neck region are summarized in Table 2.

## TREATMENT

The majority of lesions have been initially approached by incisional or excisional biopsy, followed in some instances by chemotherapy, in other instances by radiotherapy, and in yet other instances by a combination of chemotherapy and radiotherapy.[13] The most popular chemotherapeutic combination appears to have been VAC (vincristine, actinomycin D, and cyclophosphamide), although other agents (including cisplatin, vinblastine, and bleomycin) have been employed, either alone or in combination with VAC therapy.

## OUTCOME

As Table 1 demonstrates, while some patients were successfully treated by a variety of modalities, the development of a YST in the head and neck region is nevertheless a serious matter, as an appreciable number of patients (12 of the 25 patients in whom some follow-up data were available) died of their disease. Moreover, those who succumb to their disease usually do so in a disconcertingly short time — one measured in weeks to months, rather than in years. The YST has the capacity to metastasize to lung, liver, regional lymph nodes, and bone. It appears as though those patients who have documented metastases at the time of initial presentation have only a limited likelihood of responding even to heroic attempts at salvage with combined chemotherapy and radiotherapy.

With regard to the postbiopsy diagnosis planning for definitive therapy, the following generalizations may be advanced. A combination of plain films, CT, and magnetic resonance scanning may be employed to define the extent of the lesion and its relationships to neighboring structures. Serum α-fetoprotein determination will be helpful both in confirming the diagnosis of a YST and as a baseline value to follow as a gauge of the success of subsequent therapy. A combination of radionuclide bone scanning and chest CT scanning to assess for the presence of metastatic deposits may prove to be effective. Finally, it appears as though consultation with the oncologists who may be called upon to administer chemotherapy, as well as the radiotherapists, in order to judge the relative merits of preoperative and/or postoperative application of one or both of these adjuvant modalities (questions that at present are unsettled) would be wise.

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606-010-6862A

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